Emergence of Rules in Cell Society: Differentiation, Hierarchy, and Stability

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Abstract

A dynamic model for cell differentiation is studied, where cells with internal chemical reaction dynamics interact with each other and replicate. It leads to spontaneous differentiation of cells and determination, as is discussed in the isologous diversification. Following features of the differentiation are obtained: (1)Hierarchical differentiation from a "stem" cell to other cell types, with the emergence of the interaction-dependent rules for differentiation; (2)Global stability of an ensemble of cells consisting of several cell types, that is sustained by the emergent, autonomous control on the rate of differentiation; (3)Existence of several cell colonies with different cell-type distributions. The results provide a novel viewpoint on the origin of complex cell society, while relevance to some biological problems, especially to the hemopoietic system, is also discussed.

1 Introduction

A multicellular organism is an ordered clone of a fertilized egg. All the cells contain the same genome set but are specialized in different ways. The emergence of different cell types is determined rather precisely, while the developmental process of the cells, viewed as a cell society, has robustness against perturbations.

In molecular biology, the differentiation processes are often regarded as on-off switching processes. Switch depends on inputs by signal molecules, which leads to a variety of cell types as outputs. A large number of reactions between inputs and outputs are represented as a "cascade", where the reactions are assumed to be approximately independent of the other reaction processes in the cell. The switching behavior (given by the sigmoidal function) is assumed to be generated from a chain of these reactions. With this viewpoint, one can decompose the differentiation by successive local elementary processes. It enables us to elucidate the differentiation processes by experimental methods, where several signal molecules and essential genes for differentiations are identified.

Of course, the development progresses through cooperation of several processes. Successive differentiation processes are often expressed as "canalization", where differentiations are captured as a result of dynamics of complex

chemical networks, in contrast with a linear combination of simple pathways of chemical reactions. The pioneering study of Kaufmann (1969) demonstrated that the Boolean network of genes gives a variety of final states depending on the initial conditions, and he has suggested that each final state corresponds to each cell type. However, needless to say, a single initial state embedded in a fertilized egg can produce several different cell types. Thus, the following questions remain unanswered about the gene network: How do the different initial states leading to the different cell types arise in the process of development? How does a selection of specific initial conditions lead to precise rules of differentiations?

It should be noted that in the gene network picture cellular interactions are not explicitly taken into account, which should be important in the course of development. A pioneering study for the pattern formation is due to Turing (1952), where dynamic instability by the cellular interactions leads to the pattern formation (Newman, 1990). However, it remains still unsolved how such cell-to-cell interactions are incorporated with internal dynamical complexity including the gene networks (see also Bignone, 1993; Mjolsness et al., 1991; and Thomas et al, 1995).

Hence it is necessary and important to consider a system of internal dynamics with suitable cell-cell interactions. One of the authors (KK) and Yomo have performed several simulations of interacting cells with internal biochemical networks and cell divisions that lead to the change in the number of degrees of freedom. The "isologous diversification theory" is proposed as a general mechanism of spontaneous differentiation of replicating biological units (Kaneko & Yomo, 1994, 95, 97). In the theory, the following three points are essential.

- **Spontaneous differentiation:** The cells, which have oscillatory chemical reactions within, differentiate through interaction with other cells. This differentiation is provided by the separation of orbits in the phase space. The dynamics of cells first split into groups with different phases of oscillations, and then to groups with different compositions of chemicals. These differentiations are not caused by specific substances, but are triggered by the instability brought about by nonlinear systems. The background of this lies in the dynamic clustering in globally coupled chaotic systems (Kaneko, 1990, 91, 92).

- **Inheritance of the differentiated state to the offspring:** Each differentiated state of a cell is preserved by the cell division and transmitted to its offspring. Chemical composition of a cell is recursively kept with respect to divisions. Thus a kind of "memory" is formed, through the transfer of initial conditions (e.g., of chemicals). By reproduction, the initial condition of a cell is chosen so that the same cell type is produced at the next generation.

- **Global stability:** Multicellular organism often shows a robustness against some perturbation, such as somatic and other mutations. An extreme example is seen in a mutation to triploid in newt, where the cell size becomes three times, but the total cell number is reduced to one

third, and the final body remains not much affected by the mutation (Fankhauser, 1995).

The distribution of cell types obtained is robust against external perturbations. For instance, when the number of one type of cells is decreased by external removal, the distribution is recovered by further differentiations to generate the removed cell-type. In this theory, although the instability triggers the differentiations, the cell society as a whole is stabilized through cell-to-cell interactions.

In the present paper we extend these previous studies (Kaneko & Yomo, 1997) to incorporate the formation of a complex cell society. We extend our model to allow for complex internal dynamics, in particular, focusing on the following three topics.

- **(i) The hierarchical organization and the emergence of stochastic rules;** The cell differentiation process in nature follows a hierarchical organization. For example, the pluripotent cells like stem cells give rise to committed cells, which further differentiate to terminally differentiated cells. Here the rules of differentiation are written as expressions of DNA in principle, but it should be noted that the differentiation is often interaction dependent. Furthermore, in the hemopoietic system, the differentiation process appears to be stochastic, and the probability of each choice seems to depend also on the distribution of cell types (Ogawa, 1993). Hence it is interesting how such interaction-dependent rules of hierarchical differentiation are formed naturally through the interplay between internal dynamics and cell-to-cell interactions.

- **(ii) Stability of cell types and cell groups;** Cells belonging to the same cell-type also slightly differ each other. Hence discretization of states to types and their continuous change coexist among cells. The differentiation rules of cell types are written for the discrete types. When cell differentiation is determined, memory of the discrete state is stable against cell division. Then, the state has to be dynamically stable (like an attractor), while for stem cells or undetermined cells, their state must have both stability and variability (differentiability) by divisions. Here we are interested in how such stability and differentiability are compatible as a state of dynamical systems. Besides the stability of a cellular state, stability about the distribution of cell types has to be attained through the developmental process. For example, the distribution of cell types in the hemopoietic system is robust against external perturbations. Here we try to answer the question of stability through an interplay of internal dynamics and cell-cell interaction, that leads to modulation of internal cellular states and to stability of distribution of the cells at the ensemble level.

- **(iii) Differentiation of cell colony;** In an organism, there often appears a higher level of differentiations, leading to several distinct types of tissues. They consist of different types of cells and/or different distribution of cell types. Indeed in the hemopoietic system, several

colonies consisting of different cell types appear from same stem cells (Nakahata et al., 1982). It is an important question how a single cell can form such different cell colonies. This is a higher level question than cell differentiation, since the population of cell types has to be differentiated.

In the present paper we study the above three problems by extending the previous model of cell differentiation, to allow for complex internal dynamics. Here the cellular states are given by a set of chemical concentrations, while the internal dynamics is given by mutually catalytic reaction networks. In contrast with the previous model, the internal dynamics allows for chaos and also coexistence of multiple attractors. Interaction among cells is given by the diffusive transport of chemicals between each cell and a homogeneous environment. Cell volume is increased through the transport of chemicals from the environment, which leads to the cell division when it is larger than a given threshold.

By allowing complex dynamics at the internal cell level, we will show that the above three problems are answered from our standpoint. First, hierarchical differentiation of several cell types is formed. There appears a cell type that plays the role of "stem cell", from which different cell types are differentiated. The probabilistic switch of cell types is given through the internal dynamics, whose rate is dependent on the interaction, and accordingly, on the distribution of other cell types. Second, the stability of cell types is given as a "partial attractor" (to be discussed) of the internal dynamics, stabilized through interactions. Third, stochastic population dynamics of cell types emerges as a higher level. It is found that this dynamics has several attracting states, which supports different stable cell colonies (tissues).

The organization of the paper is as follows. In §2, our model is presented. Although a specific type of catalytic reaction network is adopted in the present paper, it should be noted that the results are generally seen in a variety of reaction networks. Although the interaction we adopt here is global, in the sense that all cells interact with each other, our main conclusion on the differentiation and the formation of cell society is invariant even if the "spatial" effect is explicitly taken into account as local diffusion process. In §3, we will show numerical results of the evolution of cell society from a single cell, where the emergence of distinct cell types is given. Differentiation of these cells is found to obey a specific rule, that emerges as a higher level than the chemical reaction rules we have adapted. The mechanism of discretization of states, and the formation of (interaction-dependent) cell memory is discussed in §4. The rule of "stochastic" differentiation from a stem-type cell is studied in §5, where the stability of the population of cell types is noted. In §6, the diversity of cell colonies is shown in relation with several attracting states of a higher-level dynamics, i.e., the population dynamics of cell types. Summary and discussions are given in §7 and §8, where relevance of our results to cell biology is discussed, which covers origin of stem cells, in particular stochastic branching, stability and diversity of cell colonies in the hemopoietic system, and the origin of multicellular organism.

2 Model

Our model for differentiation consists of

- Internal dynamics by biochemical reaction network within each cell
- Interaction with other cells through media: Inter-cellular dynamics
- Cell division

The basic strategy of the modeling follows the previous works (Kaneko & Yomo, 1997), although we take different dynamics for each of the above three processes. In essence we assume a network of catalytic reactions for internal dynamics that allows for a periodic and/or chaotic oscillations of chemicals, while the interaction process is just a diffusion of chemicals through media.

We represent the internal state of a cell by k chemicals' concentrations as dynamical variables. Cells are assumed to be in surrounding media, where the same set of chemicals is given. Hence the dynamics of the internal state is represented by a set of variables $x_i^{(m)}(t)$, the concentration of the m-th chemical species at the i-th cell, at time t. The corresponding concentration of the species in the medium is represented by a set of variables $X^{(m)}(t)$. We assume that the medium is well stirred by neglecting the spatial variation of the concentration, so that all cells interact each other through identical environment.

2.1 Internal chemical reaction

Within each cell, there is a network of biochemical reactions. The network includes not only a complicated metabolic network but also reactions associated with genetic expressions, signaling pathways, and so on. In the present model, a cellular state is represented by the concentrations of k chemicals.

As internal chemical reaction dynamics we choose a catalytic network among the k chemicals. Each reaction from the chemical i to j is assumed to be catalyzed by the chemical ℓ, which is determined randomly. To represent the reaction-matrix we adopt the notation $Con(i, j, \ell)$ which takes unity when the reaction from the chemical i to j is catalyzed by ℓ, and takes 0 otherwise. Each chemical has several paths to other chemicals, which act as a substrate to create several enzymes for other reactions. Thus these reactions form a complicated network. This matrix is generated randomly before simulations, and is fixed throughout the simulation. We use the same reaction-matrix throughout a series of simulations in this paper (see also §3 and §7 for dependence on the reaction-matrix).

Usually, chemical kinetics with enzymes is solved under some approximations, like Michaelis-Menten form. In this paper, we assume quadratic effect of enzymes. Thus the reaction from the chemical m to ℓ aided by the chemical j leads to the term $e_1 x_i^{(m)}(t)(x_i^{(j)}(t))^2$, where e_1 is a coefficient for chemical reactions, which is taken identical for all paths. The quadratic effect of enzymes is not essential to our scenario of cell differentiations. Several other forms on the internal dynamics lead to qualitatively the same behavior, as long as nonlinear oscillation is included. The scenario of the differentiation

which we propose here is independent of the details of this specific choice of biochemical dynamics.

Besides the change of chemical concentrations, we have to take into account the change of the volume of cell. The volume is now treated as a dynamical variable, which increases as a result of transportation of chemicals into the cell from the environment. Of course, the concentrations of chemicals are diluted according to the increase of the volume of the cell. For simplicity, we assume that the volume of cell is proportional to the sum of chemicals in the cell. Under this assumption, the operation which compensates the concentration of chemicals with the volume change is identical to imposing the restriction $\sum_\ell x_i^{(\ell)} = 1$, namely normalizing the chemical concentrations at each step of the calculation, while the volume change is calculated from the transport as will be given later.

2.2 Interaction with other cells through media

Each cell communicates with its environment through transport of chemicals. Interactions between cells, thus, occur through the environment. Here, the environment does not mean external environment for individual organism, but is intended as interstitial environment of each cell. In this model, we consider only diffusion process through the cell membrane. Thus, the rates of chemicals transported into a cell are proportional to differences of chemical concentrations between the inside and the outside of the cell. Of course, the transport through the membrane is not so simple, including several mechanisms such as channel proteins and endocytosis. We omit these complicated mechanisms for simplicity.

The transportation or diffusion coefficient should be different for different chemicals. Here we assume that there are two types of chemicals, those which can penetrate the membrane and which can not. We use the notation σ_m, which takes 1 if the chemical $x_i^{(m)}$ is penetrable, and 0 otherwise.

To sum up all these process, the dynamics of chemical concentration in each cell is represented as follows:

$$dx_i^{(\ell)}(t)/dt = \delta x_i^{(\ell)}(t) - (1/k)\sum_{l=1}^{k} \delta x_i^{(\ell)}(t) \tag{1}$$

with

$$\begin{aligned} \delta x_i^{\ell}(t) \;=\; & \sum_{m,j} Con(m,\ell,j)\; e_1\; x_i^{(m)}(t)\; (x_i^{(j)}(t))^2 \\ & - \sum_{m',j'} Con(\ell,m',j')\; e_1\; x_i^{(\ell)}(t)\; (x_i^{(j')}(t))^2 \\ & + \sigma_\ell D(X^{(\ell)}(t) - x_i^{(\ell)}(t)) \end{aligned} \tag{2}$$

where the term with $\sum Con(\cdots)$ represents paths coming into ℓ and out of ℓ respectively. The term $\delta x_i^{(\ell)}$ gives the increment of chemical ℓ, while the second term in eq.(1) gives the constraint of $\sum_\ell x_i^{(\ell)}(t) = 1$ due to the growth of the volume. The third term in eq.(2) represents the transport between the medium and the cell, where D denotes a diffusion constant, which we assume to be identical for all chemicals. Since the penetrable chemicals in

the medium can be consumed with the flow to the cells, we need some flow of chemicals (nutrition) into the medium from the outside. By denoting the external concentration of these chemicals by $\overline{X}$ and its flow rate per volume of the medium by f, the dynamics of penetrable chemicals in the medium is written as

$$dX^{(\ell)}(t)/dt = f\sigma_\ell(\overline{X^{(\ell)}} - X^{(\ell)}(t)) - (1/V)\sum_{i=1}^{N}\sigma_\ell D(X^{(\ell)}(t) - x_i^{(\ell)}(t)) \quad (3)$$

where N denotes the number of the cells in the environment, and V denotes the volume of the medium in the unit of a cell.

2.3 Cell division

Each cell takes penetrable chemicals from the medium as the nutrient, while the reactions in the cell transform them to unpenetrable chemicals which construct the body of the cell such as membrane and DNA. As a result of chemical flow, the volume of the cell is increased by the factor $(1+\sum_\ell \delta x_i^\ell(t))$ per dt. In the present paper, the cell is assumed to divide into two almost identical cells when the volume of the cell is doubled. [1] [2]

The concentrations of chemicals in the daughter cells are almost equal to the concentrations of the mother cell. "Almost" here means that the concentrations of chemicals in a daughter cell are slightly different from the mother's. Each cell has $(1+\epsilon)x^{(l)}$ and $(1-\epsilon)x^{(l)}$ respectively with a small "noise" ϵ, a random number with a small amplitude, say over $[-10^{-6}, 10^{-6}]$. Although the existence of imbalance is essential to the differentiation in our model and in nature, the degree of imbalance itself is not essential to our results to be discussed. The important feature of our model is the amplification of microscopic differences between the cells through the instability of the internal dynamics.

2.4 Internal dynamics in single cell

Before studying the dynamics of cell society, we demonstrate a typical behavior of our model by taking only one cell and medium. In our theory, the fundamental assumption is that the internal dynamics of chemicals in the cell shows oscillation as in Fig.1. In real biological systems, oscillations are

[1] In other words, the cell divides at the time t when

$$\int_{t_b}^{t} exp(1+\sum_\ell \delta x_i^\ell(t'))dt' = 2 \quad (4)$$

is satisfied since the previous division time t_b.

[2] Embryos fall into two general categories: those in which cell division is accompanied by growth of the cells back to their former volume (as mammals and birds); those in which cell division results in cells 1/2 the previous volume (as amphibians). Although our model here adopts the division process as in mammals and birds, we have also confirmed that the present differentiation mechanism also holds for a model with amphibians-like rules, where cell division makes cell volume 1/2, and each cell interacts with neighborhood cells like gap junctions.

observed in some chemical substrates such as Ca, NADH, cyclic AMP, and cyclins (Tyson et al., 1996; Hess et al., 1971; Alberts et al., 1994). Hence it is natural to postulate such oscillatory dynamics to our model. The importance of oscillatory dynamics in cellular systems has been pointed out by Goodwin (1963).

The nature of internal dynamics by eqs.(1)-(2) depends on the choice of the reaction network, in particular on the number of paths in the reaction matrix. When the number of reaction paths is small, cellular dynamics falls into a steady state without oscillation, where a small number of chemicals is dominant while other chemicals' concentrations vanish. On the other hand, when the number of reaction paths is large, many chemicals generate each other. Then chemical concentrations take constant values (which are often almost equal). Only for medium number of reaction paths, non-trivial oscillations of chemicals appear as in Fig.1. We use such network for our simulation. It is not easy to estimate the number of paths in real biochemical data, although they may suggest the medium number (3-6) of paths as required in our simulation.

Furthermore, the behavior of dynamics depends on the number of penetrating chemicals. The number of penetrating chemicals is another control parameter for the capacity of the oscillation or differentiation. When the number of penetrating chemicals is small, e.g., only one, the rate of randomly chosen reaction networks which show oscillatory dynamics is small. On the other hand, when the number of penetrating chemicals is too large, it is also difficult to obtain the network with oscillatory dynamics.

Another relevant factor to the nature of internal dynamics is the frequency of auto-catalytic paths. Indeed, the oscillatory dynamics is rather common as the number of auto-catalytic paths is increased (see §7).

3 Differentiation Process: Numerical Results

We have performed several simulations of our model with different chemical networks and different parameters. Since typical behaviors are rather common, we present our results by taking a specific chemical network with the number of chemicals $k = 20$. [3]

As an initial condition, we take a single cell, with randomly chosen chemical concentrations of $x_i^{(\ell)}$ satisfying $\sum_\ell x_i^{(\ell)} = 1$. In Fig.1, we have plotted a time series of concentration of the chemicals in a cell, when only a single cell is in the medium. This attractor of the internal chemical dynamics is a limit cycle, whose period is longer than the plotted range in Fig.1. We call this state "attractor-0" or "type-0" in this paper. This is the only attractor that is detected from randomly chosen initial conditions [4].

[3] In this example, we do not choose reaction paths equivalently among all chemicals, but select two class of reactions randomly. One class of reactions is paths from penetrable to any other chemicals, and another is paths from any of chemicals to penetrable ones. The purpose of this selection is to enhance auto-catalytic reaction loop, and to get oscillatory reaction dynamics easily. Of course, reaction networks chosen equivalently and randomly can also show the same type of behavior to be discussed in this paper. As for relationship between auto-catalytic reactions and our scenario for differentiation, see also §7.

[4] As will be seen later, there is another attractor as a single cell state. However, this

With the diffusion term, external chemicals flow into the cell, which leads to the increase of the volume of the cell. Thus the cell is divided into two, with almost identical chemical concentrations. Chemicals of the two daughter cells oscillate coherently, with the same dynamical behavior as the mother cell (i.e., attractor-0). Successive cell divisions occur simultaneously, and the cell number increases as $1 - 2 - 4 - 8 \cdots$, up to some threshold number. At this stage, internal dynamics of each cell belongs to the same attractor (i.e., attractor-0), but the oscillations are no longer synchronized. The microscopic differences introduced at each cell division are amplified to a macroscopic level through the interaction, which destroys the phase coherence.

When the number of cells exceeds this threshold value, some cells start to show a different type of dynamics. The threshold number depends on the parameters of our model. In the present example, 2 cells start to show a different dynamical behavior (as plotted in Fig.2(a)), when the total cell number becomes 16. In Fig.2(a), the time series of the chemicals in this cell are plotted. We call the state as "partial attractor-1" (or "type-1" cell). We do not call it an attractor, since the state does not exist as an attractor of internal dynamics of a single cell. As will be discussed later, the stability of the state is sustained only through the interaction. In Fig.3(a), orbits of chemical concentrations are plotted in the phase space during the transition form type-0 to type-1. It shows that each attractor occupies distinct regimes in the phase space. These two types of cells are clearly distinguishable as digitally distinct states. Hence we interpret this phenomenon as differentiation.

As the cell number further increases, another type of cell appears, which we call type-2 here. It is again differentiated from the type-0 cell (see Fig.2(b) and Fig.3(b)). The type-0 cells have potentiality to differentiate to either "1" or "2", while some of the type-"0" cells remain to be of the same type by the division.

For some simulations (i.e., for some initial conditions), the differentiation process stops at this stage, and only three types of cells coexist. In many other simulations, however, the differentiation process continues. At this stage, hierarchical differentiation occurs. The cell type "1" further differentiates into either of three groups represented as "3", "4", or "5". The time series of these three types are shown in Fig.2(c)-(e). The internal dynamics of each type is plotted in a projected phase space in Fig.4 . The orbit of type-1 cell itinerates over the three regions corresponding to "3", "4", and "5". For example, Fig.3(c) shows a switch from type-1 to type-3 in the phase space by taking a projection different from that in Fig.3(a)(b) (note the difference of scales). It is also noted that the difference by cell types is more clearly distinguishable by chemicals with lower concentrations.

In the normal course of cell differentiation process (without external operation), cells of the types "2" and "1" reproduce themselves or further differentiate to the other cell types, but the offspring never go back to the type-0 cell. Besides the cell type-2, the cell types-3, 4, and 5 reproduce themselves without any further differentiation. Among these three types, only the cell

attractor is not observed when the initial condition is randomly chosen; in other words the basin volume for it is very small.

type-5 is an attractor by itself, while others replicate only under the presence of different types of cells. Indeed, the type-5 is rather special, whose appearance destabilizes the cell society consisting of "0", "1", and "2". Once the type-5 cell appears, all the cells will finally be transformed to this type. Whether the type-5 cell appears or not depends on the initial condition, while the cell society without the type keeps diversity of cell types (see §6).

At this stage the differentiation is determined, and cellular memory is formed as is first discussed in (Kaneko and Yomo, 1997). Accordingly we can draw the cell lineage diagram as shown in Fig.5, where the division process with time is represented by the connected line between mother and daughter cells while the color in the figure shows the cell type.

The switch of types by differentiations turns out to obey a specific rule. In Fig.6, we write down an automaton-like representation of the rule of differentiation. The node "0" has three paths; one to itself, and the others to the nodes "1" and "2". The path to itself means replication of the same cell type through division, while the other paths give the differentiation to the corresponding cell types. Fig.6 represents the potentiality of these differentiations.

Note that this differentiation is not induced directly by the tiny differences introduced at the division. The switch from one cell-type to another does not occur simultaneously with the division, but occurs later through the interaction among the cells. This phenomenon is caused by dynamical instability in the total system consisting of all cells and medium. The tiny difference between two daughter cells is amplified to yield macroscopic difference through the interaction. Our results show that these transitions are not accompanied by the cell division but occur through cell-to-cell interactions. This conclusion is consistent with experimental data, where the onset of new gene expression is not always accompanied by the cell division. According to our theory and simulations, the time lag between the cell division and the onset of new gene expressions depends on the cell-to-cell interaction, i.e., the surrounding cells. On the other hand, change in the number of degrees of freedom by division amplifies the instability in the dynamics of the total system. When the instability exceeds some threshold, the differentiations start. Then, the emergence of another cell type stabilizes the dynamics of each cell again. The cell differentiation process in our model is due to the amplification of tiny differences by orbital instability (transient chaos), while the coexistence of different cell types stabilizes the system.

4 State Discretization, Hierarchical Organization and Dual Memory

One might wonder that our definition of types is rather ambiguous and is not clearly defined. Indeed one can clearly distinguish them by plotting and comparing the time series and check how these orbits are separated. To confirm that the state in each type is clearly separated, we introduce the distance between cells in the k-dimensional phase space.

Since a cell's state is determined by chemical concentrations in the present model, the cellular state is represented by an orbit in the k-dimensional phase

space. Here we first consider the average position of an orbit for simplicity;

$$\overline{x_i^{(\ell)}} = (1/T) \int x_i^{(\ell)}(t) dt \tag{5}$$

As the difference between two cells we adopt the Euclid distance

$$D_{i,j} \equiv \sqrt{\sum_{\ell} (\overline{x_i^{(\ell)}} - \overline{x_j^{(\ell)}})^2} \tag{6}$$

The distance between two cell types is plotted in Table I. Note that there remains some difference in the same type of cell as mentioned. However, this difference is clearly much smaller than that between different cell types. This demonstrates that the differentiated cell types (from "0" to "5") are well-defined as "digitally" distinct states. Then one might suspect that these different states may be just a different attractor in each dynamics. This is not the case. Except the type-0 and type-5 cells, the state of differentiated cells is unstable by itself. When we start the simulation of a single cell with the state of cell type "1","2","3",or "4" with the same media (but without any other cells), the cell is de-differentiated back to the attractor-0. The states for types "1","2","3", and "4" are stabilized only through the interaction among other cells. For example, the existence of type-0 cells is necessary to keep the stability of cell types "1" and "2".

It is also interesting to compare the bifurcation rule of cell types (in Fig.6) with the distance. If the history of cell lineage reflects on the distance of cell features, it is expected that for $j = 3, 4, 5$ $D_{1,j} < D_{0,j}$ or $D_{1,j} < D_{2,j}$ since the types 3,4,5 are derived from the type-1 cell. This is not necessarily true in Table I. The reason for this discrepancy is due to the insufficiency in the representation for the distance measured after taking the average. As is seen in Fig.4, the orbit of the type-1 cell itinerates over the states close to the type 3,4, and 5. Hence it is useful to define the minimal distance by

$$D_{i,j}^{min} \equiv min_t \left(\sqrt{\sum_{\ell} (x_i^{(\ell)}(t) - x_j^{(\ell)}(t))^2} \right) \tag{7}$$

where min_t means the minimum over time. The distance is given in Table II, where one can clearly see the hierarchical organization of cell types according to the bifurcation rule of Fig.6. The distance between two of cell types "0","1", and "2" is smaller than that between "0" or "2" and "3","4",or "5". The distance between "1" and "3","4", or "5" is much smaller than others.

Let us reconsider the form of memory using the distance. First, the memory of cell types is sustained in the internal dynamics modulated by the interaction. The memory corresponds to a partial attractor stabilized by the interaction. Here, the information on the distribution of cell types is embedded in each internal dynamics.

For example, each internal dynamics is gradually modified with the change of distribution of other cells. In Fig.7 we have studied how the dynamics of the type-2 cell changes when the rate of type-0 cell is varied. In the simulation, we choose (a stable) cell society consisting of types "0", "2", and "3", and successively replace a cell of type-0 by type-3. To avoid the perturbation due to the change in the number of cells, we remove the rule of

division in the present simulation, to fix the number. As a result of change of the distribution of cell types (i.e. the fraction of type-0 cells), the dynamics of each cell (e.g., of the type 2) is modulated. We have plotted the distance $D_{2,2_0}$ where the 2_0 denotes the cell when the distribution of cells satisfies $(n_0, n_2, n_3) = (23, 50, 27)$, the condition at the left-end point of the axis, where n_k represents the number of the type-k cell. The distance $D_{2,2_0}$ increases (roughly linearly) with the decrease of n_0, until the further decrease destabilizes the cell society and the switching of cells to type-5 starts. The gradual change of $D_{2,2_0}$ means that the internal cell state varies according to the cell distribution. Hence the global information on the cell distribution is embedded in the internal cellular state. We note that this information adopts "analogue" representation, instead of digital one adopted for the distinct cell type. Hence our cellular system has both analogue and digital memories.

5 Interaction-dependent rules and stability of cell society

In Fig.6, we have shown that the automaton-like rule has emerged without explicit implementation. The rule is not solely determined by its cell type. When there are multiple choices of differentiation process (as in "0" $\rightarrow$ "0", or "0" $\rightarrow$ "1", and "0" $\rightarrow$ "2") the rate of each path is neither fixed nor random, but depends on the number distribution of cell types in the system, embedded in the internal dynamics. This implies that a higher level dynamics emerges, which controls the rate of cell division and differentiation according to the number of each cell type. In other words, the dynamics on the number of each cell type n_0,n_1,.., and n_5 can be represented by $\{n_k\}$ $(k = 0, \cdots, 5)$. (This dynamics should be stochastic, since we have neglected the information on each cellular state and reduced it to only the number of cell types).

This dynamics allows for stability at the level of ensemble of cells. The variety and the population distribution of cell types are robust against external perturbations. As an example, let us consider the case with three cell types ("0" ,"1" ,"2" in Fig.6). When the type-2 cells are removed to decrease their population, events of differentiations from "0" to "2" are enhanced, and the original cell-type distribution is recovered.

In Fig.8, the rate of differentiation from the type-0 cell to others is plotted. In this simulation, to capture the dynamics of the number of each cell type, the total number of cells in the medium is fixed (to N=100 in the present case), by removing the division rule. As the initial condition, N cells are placed in the medium, where the concentration of chemicals in each cell is selected so that they give type-0, 1, or 2 cell. The switch of cell types is measured when the system settles down to a stable distribution of cell types. The simulations are repeated by changing the initial distribution of cell types (n_0, n_1, n_2), to plot the number of the switches from 0 to others, while the final number of cells for each type is also plotted. As in Fig8, the frequency of switches from the cell type-0 increases almost linearly with n_0 when it is larger than approximately 40%. With this switch, the stability of cell distribution around approximately $(n_0, n_1, n_2) = (40, 30, 30)$ is attained.

This kind of robustness at an ensemble level is expected from our isolo-

gous diversification theory, since the stability of macroscopic characteristics is attained in coupled dynamical systems (Kaneko 1992, 94). In our case, the macroscopic stability is sustained by the change of the rate of differentiation from "0" to other types. Recall that, the differentiations from "1" or "2" to "0" does not occur (see Fig.6), even if some of the type "0" cells are removed [5]. In the hierarchical structure represented in Fig.6, the cell at an upper node behaves as a stem cell, and regulates the distribution of the cells at a lower node. This type of regulation system is often adopted in the real multicellular organism (e.g. in the hemopoietic system)(Schofield et al., 1980). An important point of our result is that the dynamical differentiation process always accompanies this kind of regulation process, without any sophisticated programs implemented in advance. This robustness provides a novel viewpoint to understand how the stability of the cell society is maintained in the multicellular organism.

6 Differentiation of Colonies

The automaton rule of Fig.6 does not necessarily mean that all of these six types of cells coexist in a cell society emerged in the course of the development. Cell groups consisting only of two or three cell types can appear: For example, cell groups only of "0","1", and "2" types and of "0", "2", and "4" types are observed.

This implies that the dynamics on the number of cells of each type has also several stable attractors due to the autonomous control of the rate of differentiation. They correspond to stable distributions of cell types in each cell group. In other words, there are several possible distributions of cell types when cells are developed from a single cell. To confirm it, we have performed the following simulations. First, we initially put one cell whose internal chemical concentrations are chosen randomly. Then the cell society is evolved following the rules of the present model, until the total cell number reaches a given threshold value, when we stop the simulation and measure the distribution of cell types. We have repeated this course of simulations for hundred times, starting from different initial conditions.

In Fig.9, the number of initial configurations leading to a cell-type distribution with a given range of n_2 is plotted as a histogram, where the number of type-2 cells n_2 is measured when the total cell number has reached 300. Four peaks are clearly visible at $n_2 = 0$,$\sim$ 100, $\sim$150, and $\sim$220, which correspond to possible distinct sets of cell distributions. As mentioned, the possible set of cell types (from "0" to "5") and the temporal ordering of their appearance (e.g., $0 \to 1 \to 2$) are independent of the initial conditions. However, at the later stage, several types of cell groups emerge depending on the initial conditions.

The most relevant factor to the choice of cell groups is the ratio of the numbers of differentiated cells (i.e. type-1 and type-2 cells) to undifferentiated cells (i.e. type-0 cells) at an early stage of development, when the first

[5]Transformation from type-2 to type-0 cells occurs as a transient process to type-5 cell, which is seen only in the case when the type-5 cell appears and starts to dominate the society.

differentiations from "0" to "1" and "2" occur. Thus the fate of cell groups is determined at a rather early stage.

Recall that the differentiation rate of cells (each arrow in Fig.6), and accordingly the higher-level dynamics of n_k depend on the distribution of cells n_k. The result of Fig.9 implies that there are several attractors on this higher-level dynamics on n_k. As discussed in §5, an "attractor" of this higher level dynamics is stable against perturbations to change the number of cells of each type.

In Fig.10 we have shown the flow chart of the change of (n_0, n_1, n_2), where the direction of change of n_0 and n_2 is represented by the arrow, starting from the initial distribution given by (n_0, n_1, n_2) of the corresponding site. To draw the figure, we adopt the same rules as in Fig.8, where the total cell number $(n_0 + n_1 + n_2)$ is fixed to 100, and the division rule is removed. From the 2-dimensional plane, the number of cells of types 1,3,4, and 5 are given by $100 - n_0 - n_2$. The chart shows that cell colonies on the cell-type distribution $\{n_0, \cdots n_5\}$ have at least 5 stable states around (n_0, n_2) =(0,0), (38,32), (30,50), (18,58), and (0,78) respectively. Each state has a basin of attraction, and the corresponding cell-type distribution is stable against external perturbations, as is supported by the higher-level dynamics on $\{n_0, \cdots n_5\}$.

The fixed point at $(n_0, n_2) = (0, 0)$ ("A" in the figure) corresponds to a colony consisting only of type-5 cells, while the fixed point denoted by "B" corresponds to a colony only of 0,1,2, "C" of 0,2,3, and "D" of 0,2,4, respectively. Indeed, these cell-type distributions correspond to the peaks of Fig.9, respectively.

Still there is a clear difference between the developmental process from one cell (Fig.9) and the present simulation (Fig.10) with a fixed cell number. Some region in the plane of Fig.10 cannot be reached by the simulation from a single cell. For example, the state "E" consisting of types 2 and 4 cannot be obtained from the developmental process from a single cell. Furthermore, the state "B", which does not have a large attraction volume in Fig.10, has the largest probability to be reached from the developmental process (see Fig.9). This discrepancy is caused by the conjunction of cell number change with the population dynamics of cell types. Through the change of the number of cells, the population dynamics shifts from one flow chart of Fig.10 to another with different number of cells. The organized cell colony from a single cell has such developmental constraints.

Now the coexistence of several stable cell colonies is clear. Depending on the initial cell condition, different cell colonies are obtained. The result here means that several types of tissues can appear through the interactions among cells. This kind of diversity is often observed in a cultivation system of a colony of blood cells starting from a stem cell (Nakahata et al., 1982).

7 Summary

In the present paper, we have studied a dynamical model to show that a prototype of cell differentiation occurs as a result of internal dynamics, interaction, and division. We have made several simulations choosing several

chemical networks, also with a different number of chemical species, and the same scenario for cell differentiation is obtained. Under the same parameters used in the previous example, approximately 5% of randomly chosen chemical networks show oscillatory behavior, while others fall into fixed points. Furthermore, approximately 20% of these oscillatory dynamics are destabilized through the cell division, where some of the cells differentiate following a specific rule like Fig.6.

Some may cast a question why we can select such oscillatory dynamics to draw a general mechanism for differentiation, even if only a few randomly chosen chemical networks are oscillatory. One reason why only a few reaction networks are oscillatory is that we choose reaction paths randomly and with the identical coefficients. On the other hand, the chemical reaction network of the real biological system is more sophisticated through evolutionary process. For example, there are positive and negative feedback reactions ubiquitously. This feedback mechanism, in particular auto-catalytic reaction, is important to provide oscillatory dynamics which are observed in the real biological system (e.g. Ca oscillation).

In the present model with randomly chosen networks, only few reactions have auto-catalytic effects. By increasing the rate of auto-catalytic reaction paths, the probability of the network with oscillatory dynamics and differentiation gets much higher. For comparison, we have also studied a class of models where each chemical can catalyze a reaction to generate itself from another chemical, besides the ordinary reaction paths determined randomly. By sampling several reaction networks, we have found that 40% of the reaction networks has oscillatory dynamics and more than 20% of these dynamics are destabilized to show cell differentiation by cell divisions.

Then, why are such auto-catalytic reactions common? To make replications efficiently, some mechanism to amplify reaction by its product is generally expected at the first stage of life (Eigen and Schuster, 1979). Also, auto-catalytic reactions are necessary to add new metabolites in the metabolic network through the evolutionary process. Indeed, when novel chemicals are included in the evolutionary process of metabolic network, their concentrations must be amplified by the reactions. This implies that these new chemicals must constitute an auto-catalytic set (see Appendix of Kaneko and Yomo, 1997).

Let us summarize the consequences of our simulations. First, we have provided a further support for isologous diversification previously proposed. Cells are differentiated through the interplay between intra-cellular chemical reaction dynamics and the interaction among cells through media. As the cell number is increased, the oscillatory dynamics in each cell is destabilized and loses synchrony. Then, some of cells change their internal dynamics, which form a group with a different stable dynamics. Discrete, differentiated states appear, which are transmitted to their daughter cells as a memory. We interpret this phenomenon as (determined) differentiation.

The differentiation in our theory is caused by the instability in internal dynamics triggered by cell-to-cell interactions. Microscopically speaking in biological terms, this may be regarded as a switching process following signal molecules from outside of the cell. Our theory is not inconsistent with such biological knowledge, but the point in our theory lies in that such local

transition of internal states has also the information on macroscopic states, i.e., the distribution of cell types. With this, the robustness of cell society emerges in spite of instability in each internal dynamics.

Besides this further support for the isologous diversification, we have demonstrated the hierarchical cell differentiation, generation of interaction-dependent rules, and the existence of distinct cell groups.

1) hierarchical organization

Differentiation from a stem cell to two different types, and then to three types from one of them are observed. Hierarchical rule of differentiation is thus generated. Although the number of cell types and the rule of differentiation depend on the choice of chemical networks, generation of a hierarchical rule (written by the tree-type diagram as in Fig.6) is generally observed.

2) generation of rules and internal memory reflecting on the environment (that is the distribution of other cell types)

These differentiations obey a specific rule, which emerges from inter- and intra- dynamics. It is often believed that the rules of the differentiation, which determine when, where, and what type of a cell appears in a multicellular organism, should be pre-specified as the information on DNA. We do not deny such role of DNA, but it should be stressed that the rules of differentiation and the higher level dynamics emerge through interaction of cells with internal dynamics. As a consequence of our interaction-based approach, the diversity of cells and the stability of cell society naturally follow.

The rate of differentiation and reproduction vary with the distribution of cell types. The global stability of the whole system is obtained, which is sustained by regulating the rates of the differentiations.

As a coupled dynamical system, the memory of cell types is given in a state stabilized by interactions. This state is not necessarily an attractor as a single cell dynamics, but is a "partial attractor" stabilized only in the presence of suitable interactions provided by the distribution of other cells. Through the cell divisions and the evolution of the cell society, the cells choose suitable interactions so that the memory of their types is preserved. This is the mechanism of how the recursivity of cell types is attained, while the global stability of cell society is assured through the interaction. Indeed such partial attractors lose stability and switch to other cell types when the interaction by the cell distribution is not "suitable".

It should be noted that two types of memory coexist, analogue and digital. The former gives information on the cell society, i.e., the distribution of cell types, while the latter gives a distinct internal state on cell differentiation. We believe that such dual memory structure is a general feature in a biological system. In cell biology, the "analogue" difference reflecting on the interaction is known as modulation (Alberts et al., 1994).

3) formation of higher level dynamics and diversity of cell groups

The rule of differentiation depends on the number distribution of other cell types, for which stochastic dynamics at a higher level is formed. The result provides the first example that a 2-step higher-level dynamics is formed, that is a colony level from cellular one, that is formed from a chemical network level. Here the dynamics of a colony level (i.e., the change of the number of each cell type) is "stochastic", because the information on the number of cell types is not complete, where the lower-level information on the internal

state (of chemical concentrations) is discarded. It is interesting to note that the macroscopic flow chart on the number of cell types is formed in spite of the stochasticity.

Our result shows that there are several attracting states for this higher-level dynamics. In biological term, this corresponds to the existence of several cell colonies, distinguishable by the number distribution of cell types. These diverse colonies appear from a single stem cell. Each cell colony is stable, in the sense that the original distribution is recovered after perturbations (of not too large size) are added on the cell colony, such as elimination of few cells of one type.

8 Discussion

Of course, there has been preceding theories for cell differentiation. The idea to regard the differentiation as the transition in cellular multi-stationarity is traced back to Delbrück (1949), who proposed a simple bistable reaction network with two metabolic chains that are cross-inhibited by their products. Indeed the epigenetic transmission of such stationary states have been reported in unicellular organisms (Novick and Wiener, 1957; Sonneborn, 1964).

In general, this multi-stationarity results from positive and negative feedbacks in metabolic reaction networks. This leads to the viewpoint that each differentiated cell state is represented by an attractor of intra-cellular dynamics, as has been demonstrated by Kauffman (1969) in his Boolean network. It leads to a variety of stable states (attractors), depending on the initial conditions. Here, each cell type corresponds to an attractor of internal networks, while an external mechanism is required to have the transition between these attractors.

Such mechanism is supported by cell-to-cell interaction. Indeed, a mechanism of interaction-induced differentiation has been proposed by Turing's pioneering study (Turing, 1952). Now, a well-known mechanism of external regulation is gradient of morphogen, in which the transition depends on the concentration of chemical substances. (See, however, Kaneko and Yomo (submitted), for instability due to stochastic fluctuation in the threshold mechanism on the gradient of chemicals). Another possible mechanism for interaction-induced differentiation is proposed by Gordon, where the mechanical wave transmits among the cells and controls the cell state (Gordon et al., 1994).

Then, combination of the multi-stationary reaction network and the external regulation mechanism might be relevant to explain the local differentiations. However, to understand the complexity and the stability of cell society, an important question still remains: How is such external regulation mechanism regulated? Is another external mechanism required?

Our results provide a distinct, and plausible standpoint for this problem. Noticing the interplay between intra-cellular dynamics and interaction, we have proposed a novel concept "partial attractor", which is stabilized only by cell-to-cell interaction. Thus, a cell state is not always determined by the attractor of internal dynamics, but it also depends on the other cells. An important consequence of our results is that there is no distinction be-

tween internal dynamics that determines the cell state and the regulation mechanism of differentiation. Rather, the mechanism for regulation is spontaneously accompanied by the multi-stationarity, because the number of cells with each partial attractor is found to depend on the circumstance of cell society which sustains them.

A consequence of our theory is 'relativity' of determination of a differentiated cell. Since our cellular state reflects the interaction, the rule of differentiation as well as the recursivity may be affected by the cells around the cell in concern. A suitable experiment system to distinguish our theory from previous theories is provided by a hemopoietic stem cell system, where spatial pattern mechanism a l'a Turing no longer works. Now we will discuss briefly relevance of our results to the cell biology.

Application to biological problems

Since our model reaction process does not have one-to-one correspondence to any existing biochemical network, one cannot make a detailed prediction on a specific example in biology. However, the present scenario sheds a new light on some open questions in biology, by providing a coherent viewpoint on them. Let us discuss two of them.

Since our results provide hierarchical differentiation, it is interesting to compare them with such an example in cell biology. A well known example is a hemopoietic system (Ogawa, 1993). The blood contains many types of cells with different functions, while a pluripotent stem cell in the bone marrow gives rise to all classes of blood cells. The hemopoietic system can be viewed as a hierarchy of cells, where pluripotent stem cells differentiate to progenitor cells determined as ancestors of one or a few terminal differentiated blood cell types. In general, these terminal blood cells have limited lifespans and are produced throughout the life of the animal. Thus, to keep a variety of blood cells, it is important to control the differentiation and proliferation of the stem cell at the higher level of hierarchy. However, because of the difficulty of identifying the stem cells in the bone marrow, the behavior of the pluripotent stem cells in vivo remains especially elusive. In the experimental result in vitro, even if the cells have been selected to be as homogeneous as possible, there is a remarkable variability in the sizes and often in the characters of the developed colonies (Nakahata et al., 1982). Even if two sister cells are taken immediately after a cell division and cultured apart under identical conditions, they frequently give rise to colonies that contain different types of blood cells or different distribution of the types of cells.

It is often interpreted that the differentiation of a hemopoietic stem cell is stochastic whose probability is controlled by some other control mechanism, by which the multicellular system as a whole regulates the distribution of cell types(Till et al., 1964; Ogawa, 1993). Results of our model provide a novel interpretation of these experiments. First, the rules of differentiation are generated through interactions. Second, the stochastic differentiation of the cells and regulation of the probability of the differentiations naturally emerge from the cell-to-cell interaction, without imposing any random event or external regulation mechanism. Third, the diversity of the colonies which have developed from a same type of cell is a natural consequence, as a multistability of higher-level dynamics. We note that a single cell with a slightly different initial condition can lead to a different colony in our simulation.

Through the chemical substrates such as the Interleukins, the complicated interactions among the blood cells are observed experimentally. It is plausible to assume the dynamical interaction adopted in our theory.

Here, we propose an experiment on the hemopoietic system to make some predictions. As mentioned, one of the important consequences of our results is that the states of cells are not always determined by the attractor of internal dynamics, but often are sustained by interaction with other cells. This implies that some types of cells in the hemopoietic system do not correspond to a stable attractor of internal dynamics, but are stabilized by other cells. The pluripotent stem cell and the terminal differentiated cell, the top and bottom of the hierarchy respectively, seem to correspond to a stable attractor, because they can stand independently of other blood cells. On the other hand, the progenitor cells can be observed only in the colonies of blood cell. We expect that the internal dynamics of these progenitor cells is represented by a partial attractor. Then, these cells can differentiate back to the cell of higher hierarchy when these cells are separated and cultured independently. To confirm this hypothesis, differences between an isolated blood cell and that surrounded by other cells should be tested experimentally. We predict that the potentiality of blood cells to differentiate and to proliferate is quite different these two situations, which confirms the importance of the cell-cell interaction in the hemopoietic system.

In general, there is a level of differentiations in cell. The determined differentiation keeps the memory even if a cell is transplanted, while some cells can be transdifferentiated (Alberts et al., 1994). In our dynamical systems representations, such difference can be expressed as the distinction between an attractor by the cell itself and the partial attractor stabilized by the interaction. The merit in our approach is that such levels of differentiation appear without external implementation, which is important when one considers the origin of multicellular organisms.

There are several types of cells in a multicellular organism. In particular, almost all organisms have distinction between the germ cells and the somatic cells. The appearance of these two types are controlled elaborately by complicated interaction among cells in the contemporary multicellular organisms. However, it is hard to postulate that such a mechanism appeared at the same time with the emergence of the multicellular organisms. Our theory provides one possible solution to this problem. According to our results, differentiation in a group of identical cells occurs through the dynamical interaction among cells, as long as the intra-cellular reaction dynamics can show nonlinear oscillations. The differentiation, as well as the stability of such diverse cells, is a rather natural consequence of interacting cells. At the next stage in the evolution, more complex cell society must have appeared, where several types of tissues exist as a higher hierarchy, which interact each other. Our result about the diversity of cell group shows potentiality that several types of tissues appear at this stage, based on the dynamical interaction among cells. In our model, we do not take into account of the spatial variation. Selection of each cell group, thus, depends on the choice of different initial conditions. On the other hand, when the spatial information is included in this system, it is possible that several types of cell group coexist at a (spatially) different region.

Of course, contemporary multicellular organisms such as mammal often have hundreds of cell types, though our results in this paper can show coexistence with only few cell types. The number of cell types in our dynamical differentiation model does not show clear increase with the number of chemical substances. We suppose that the reason for these few types of cells is due to our random choice of chemical reaction network, where the reaction paths are chosen equivalently. In real biological system, the chemical reaction network is more organized, possibly in a hierarchical manner.

To choose such suitable network, evolutionary aspect of chemical reaction network should be taken into account for our model. This problem also concerns with the emergence of gene expression system, and is under our current investigation.

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Figure caption

Fig.1: Overlaid time series of $x^{(m)}(t)$ of a single cell in medium, obtained form a network with 16 chemicals and three connections in each chemical. Only the time series of 5 chemicals are plotted out of 16 internal chemicals. Each line with the number m=2,9,10,11,12 gives the time series of the concentrations of the corresponding chemical $x^{(m)}(t)$. This oscillatory behavior is a limit cycle, whose period T is longer than the plotted range of the figure ($T \cong 16000$ time steps). The parameters are set as $e_1 = 1$, $D = 0.01$, $f = 0.01$,$\overline{X^{(\ell)}} = 0.1$ for all ℓ, and $V = 100$. Chemicals $x^{(\ell)}(t)$ for $m \leq 3$ are penetrable(i.e., $\sigma_\ell = 1$), and others are not. The reaction network $Con(i, j, \ell)$ is randomly chosen initially, and is fixed throughout the simulation results of the present paper.

Fig2: Time series of $x^{(m)}(t)$, overlaid for the 5 chemicals (as given in Fig.1) in a cell. (a)-(e) represent the course of differentiation to type-1, 2, 3, 4, and 5 cells respectively. The differentiation to type-3, 4, and 5 cells always occurs from type-1 cells.

Fig.3: Orbits of internal chemical dynamics in the phase space. (a) and (b) show the orbits of chemical concentrations for a switching process from type-0 to type-1,2 cells, respectively, plotted in the projected space $(x^{(2)}(t), x^{(13)}(t))$. Fig.3(c) gives a plot of $(x^{(1)}(t), x^{(8)}(t))$, which shows a switch from type-1 to type-3 cells. (Note the difference of scales.) Each cell type is clearly distinct in the phase space.

Fig.4: Orbits of internal dynamics for each cell type. The dynamics of each cell type is plotted in the same projected space as in Fig.3(a),(b), i.e., $(x^{(2)}(t), x^{(13)}(t))$. Color corresponds to each cell type.

Fig.5: Cell lineage diagram. Differentiation of cells (whose indices are given by the horizontal axis) is plotted with time as the vertical axis. In this diagram, each bifurcation of lines through the horizontal segments corresponds to the division of the cell, while the color indicates the cell type (red for type 0, green for type 1, blue for type 2 respectively.). We have plotted up to the stage of the differentiation to three types, while the bifurcation to 3,4, and 5 from 1 occurs at a later course.

Fig.6: Automaton-like representation of the rule of differentiation. The path returning to the node itself represents the reproduction of its type, while the paths to other nodes represent the potentiality to differentiation to the corresponding cell types. The dotted line from type-2 to type-0 gives an exceptional case: Indeed the differentiation from "2" to "0" never occurs when several types of cells such as "0","1" and "2" coexist. It occurs exceptionally only if "5" cells dominate the system, when all cells are finally differentiated to type-"5". In this case the type-"2" cells de-differentiate to "0" (and finally to "5").

Fig.7: Variation of dynamics of type-2 cells with the change of the rate

of type-0 cell. The distance $D_{2,2_0}$ of eq. (6) is measured between two type-2 cells from the conditions $(n_0, n_2, n_3) = (23, 50, 27)$ and $(n_0, n_2, n_3) = (n_0, 50, 50 - n_0)$,as one of type-3 cells is successively switched to type-0 externally by 2×10^4 step. Besides the distance, the number of type-0 cells is plotted against time.

Fig.8: Rate of the differentiation from type-0 to other cell types. The total cell number is fixed to 100 (without division process), while we take the initial cell distribution of three types as $(n_0, n_1, n_2) = (n_0, 30, 70 - n_0)$. Starting the simulation with this initial condition, the final number of each cell type, as well as the number of differentiations from type-0 to others, is plotted, as a function of the initial number of type-0 cells (n_0). Within this range of cell type distribution, none of type-3,4,5 cells appear(see section 6).

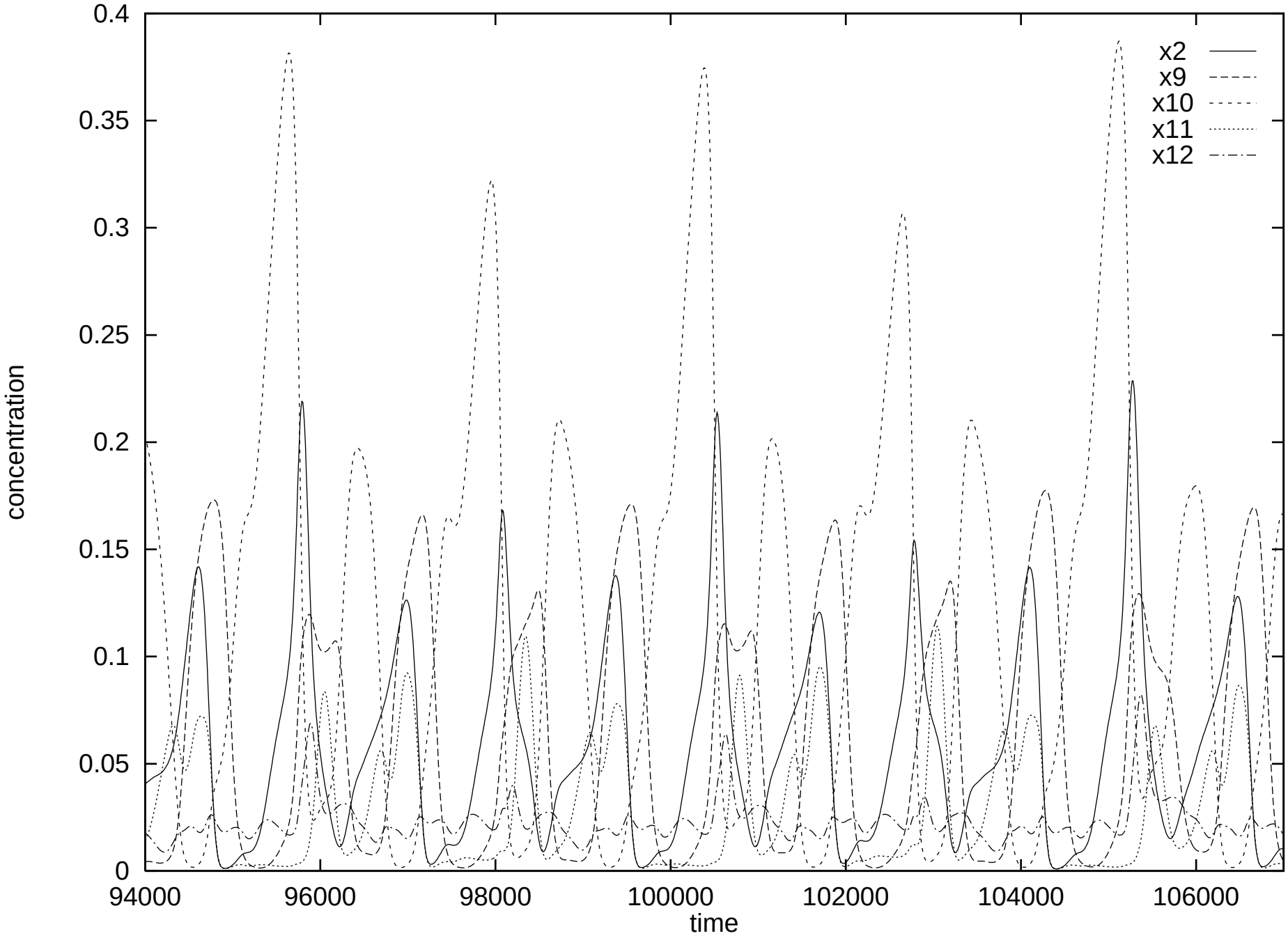
Fig.9: Histogram about the number of type-2 cells. Starting from a single cell with randomly chosen chemical concentrations, the simulation is carried out until the total cell number reaches 300, when the number n_2 of type-2 cells is measured. Repeating the runs 347 times, we have counted the number of such initial conditions that n_2 falls onto a given bin (with the size 5). The histogram of n_2 is obtained from the count. There are four peaks at n_2=0,100,150,220, each of which corresponds to a stable distribution of the cell colony.

Fig.10: Flow chart of the change of (n_0, n_1, n_2). We have carried out the simulations starting from the initial condition at each $(n_0, 100 - n_0 - n_2, n_2)$ by fixing the total cell number to 100 (by removing the cell division process). Change of the number of cell types is measured from simulations, from which the direction of changes of (n_0, n_2) is shown as an arrow in the (n_0, n_2) space. As is seen, there are 5 fixed points, each of which corresponds to the stable population distribution of cell types.

Table Caption

Table I: The average distance in phase space between each cell type: $D_{i,j}$ in eq.(6) is estimated by taking the average over 5×10^4 time steps. Each cell type is sampled from a course of the evolution starting from one cell.

Table II: Minimum distance in phase space between each cell type. $D_{i,j}^{min}$ in eq.(7) is estimated from 5×10^4 time steps. Each cell type is sampled from a course of the evolution starting from one cell.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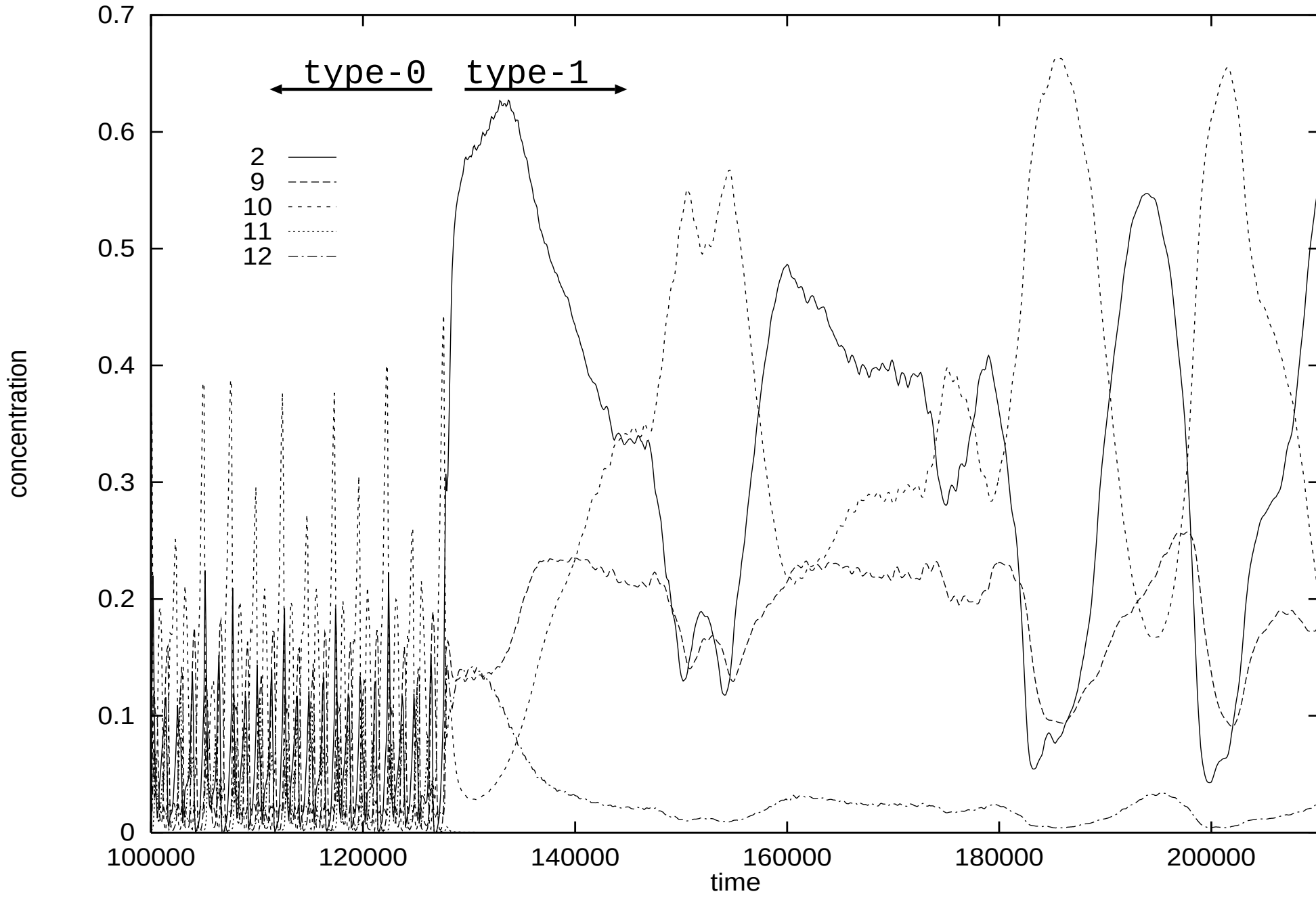
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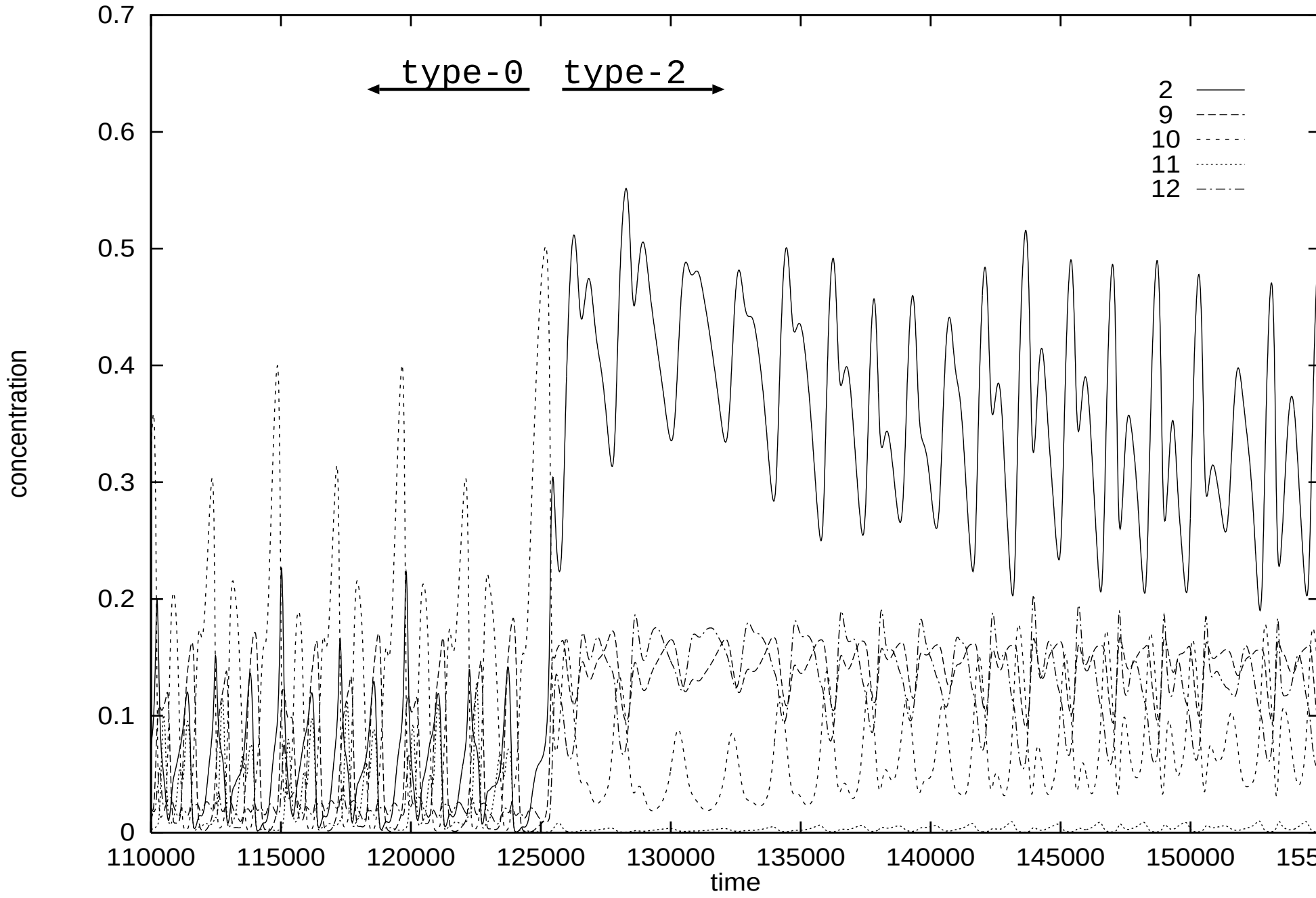
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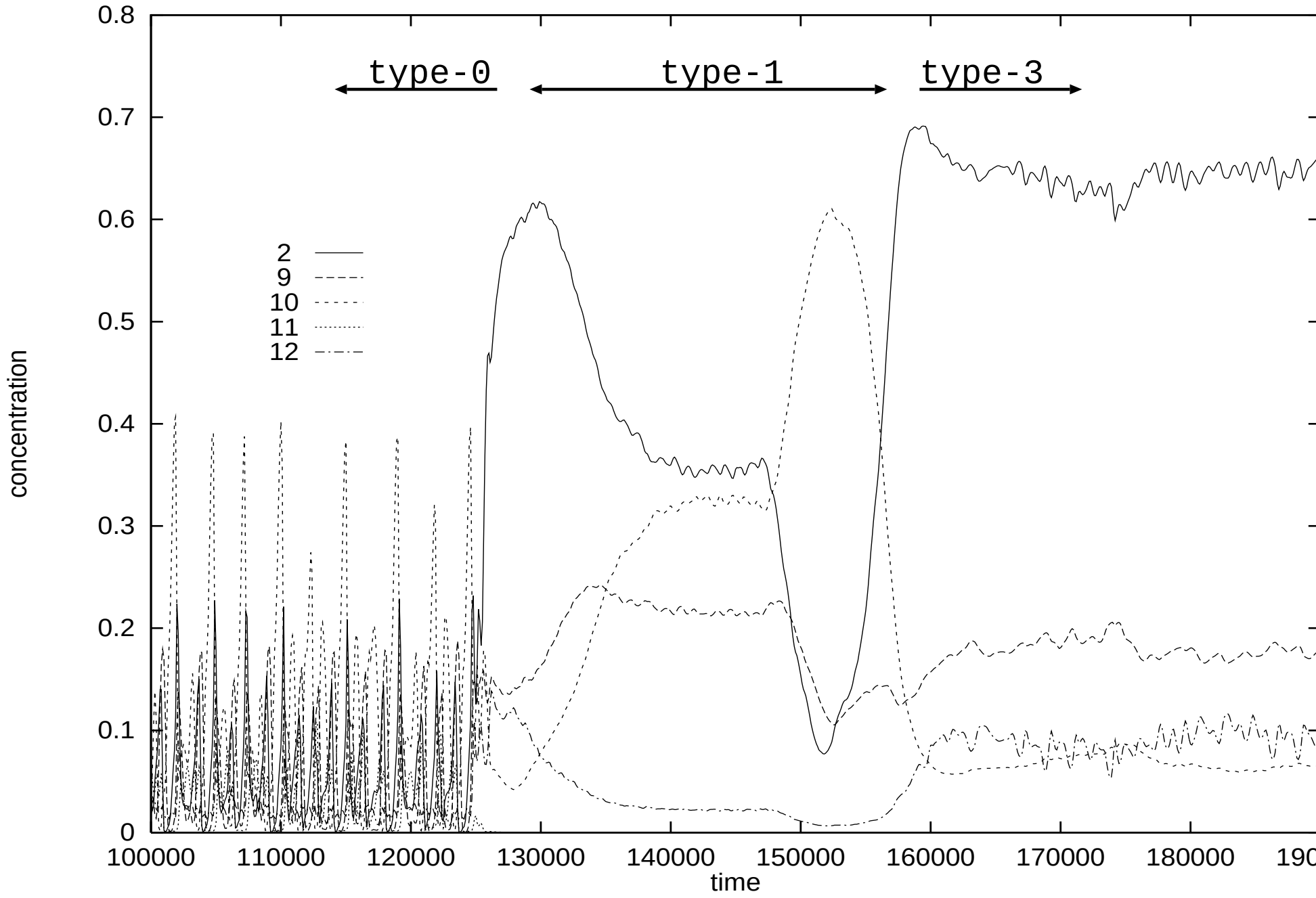
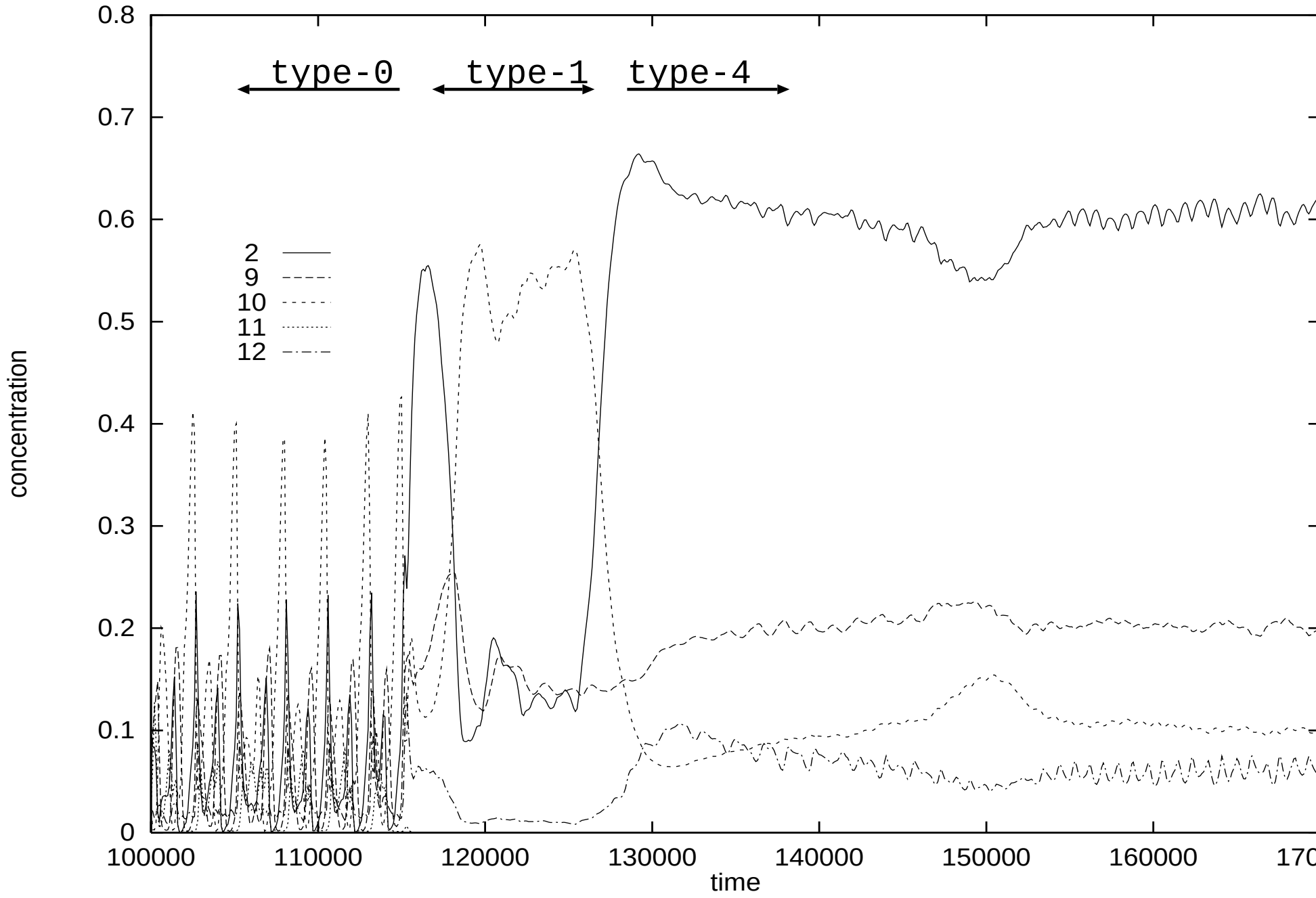
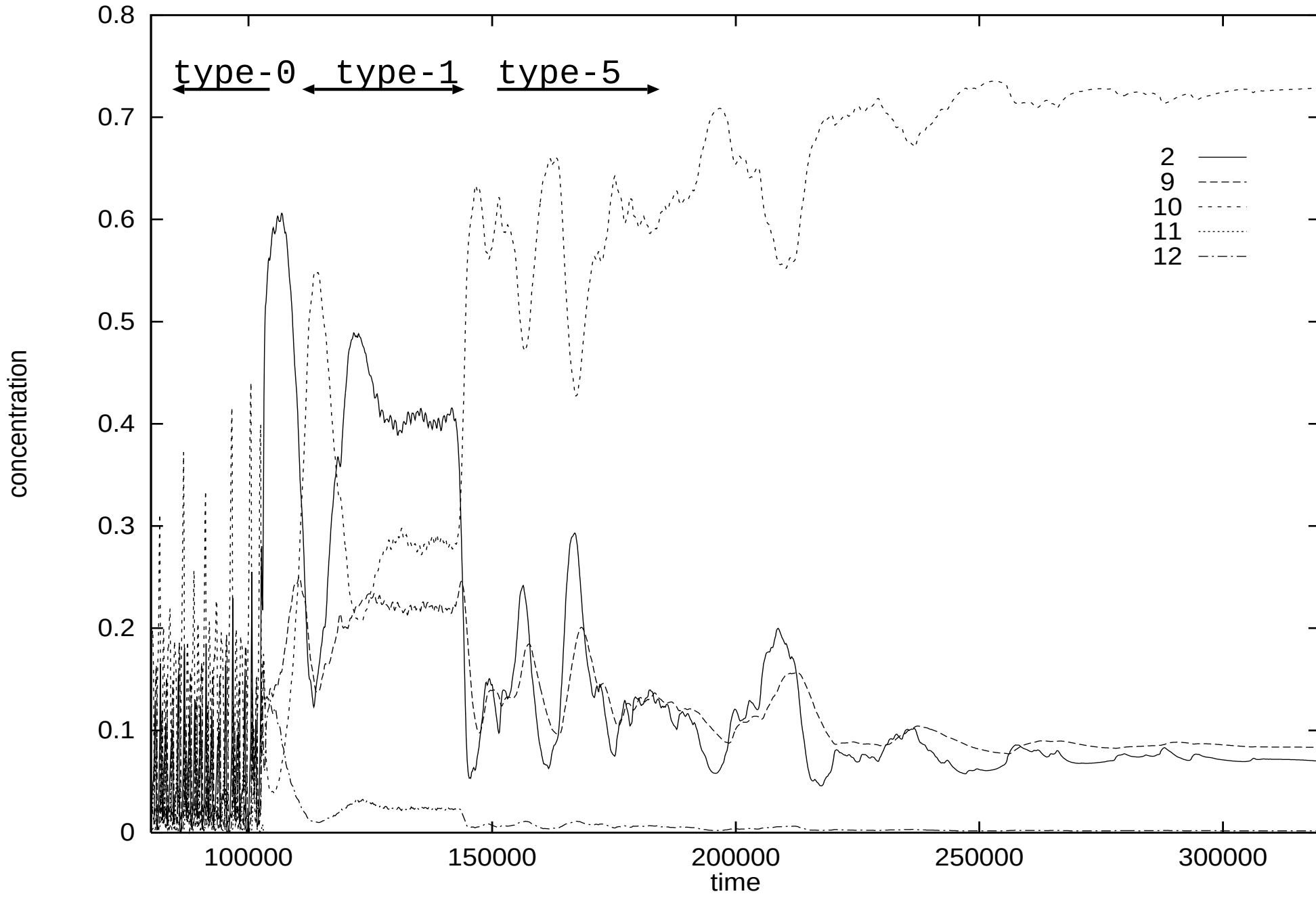
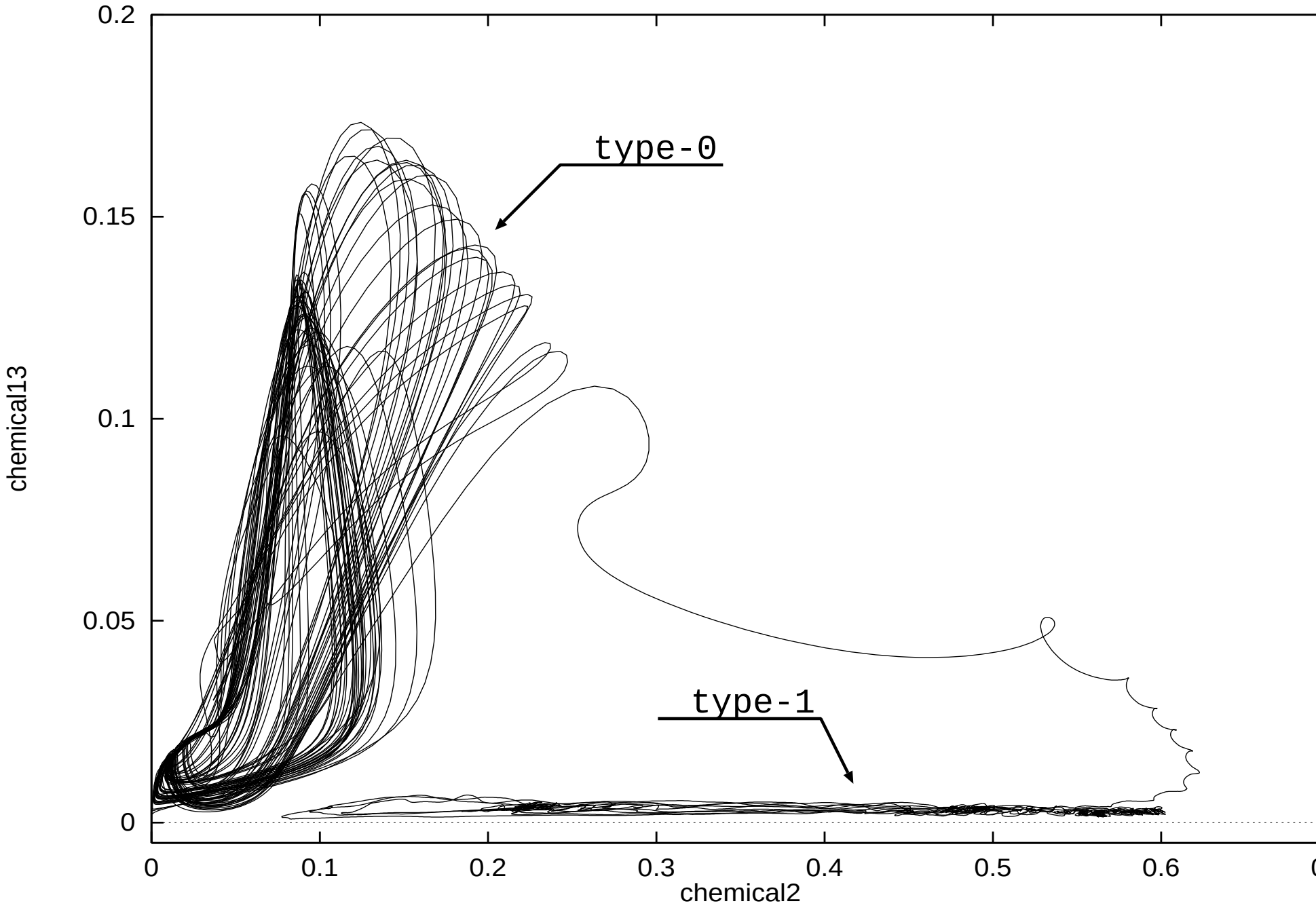
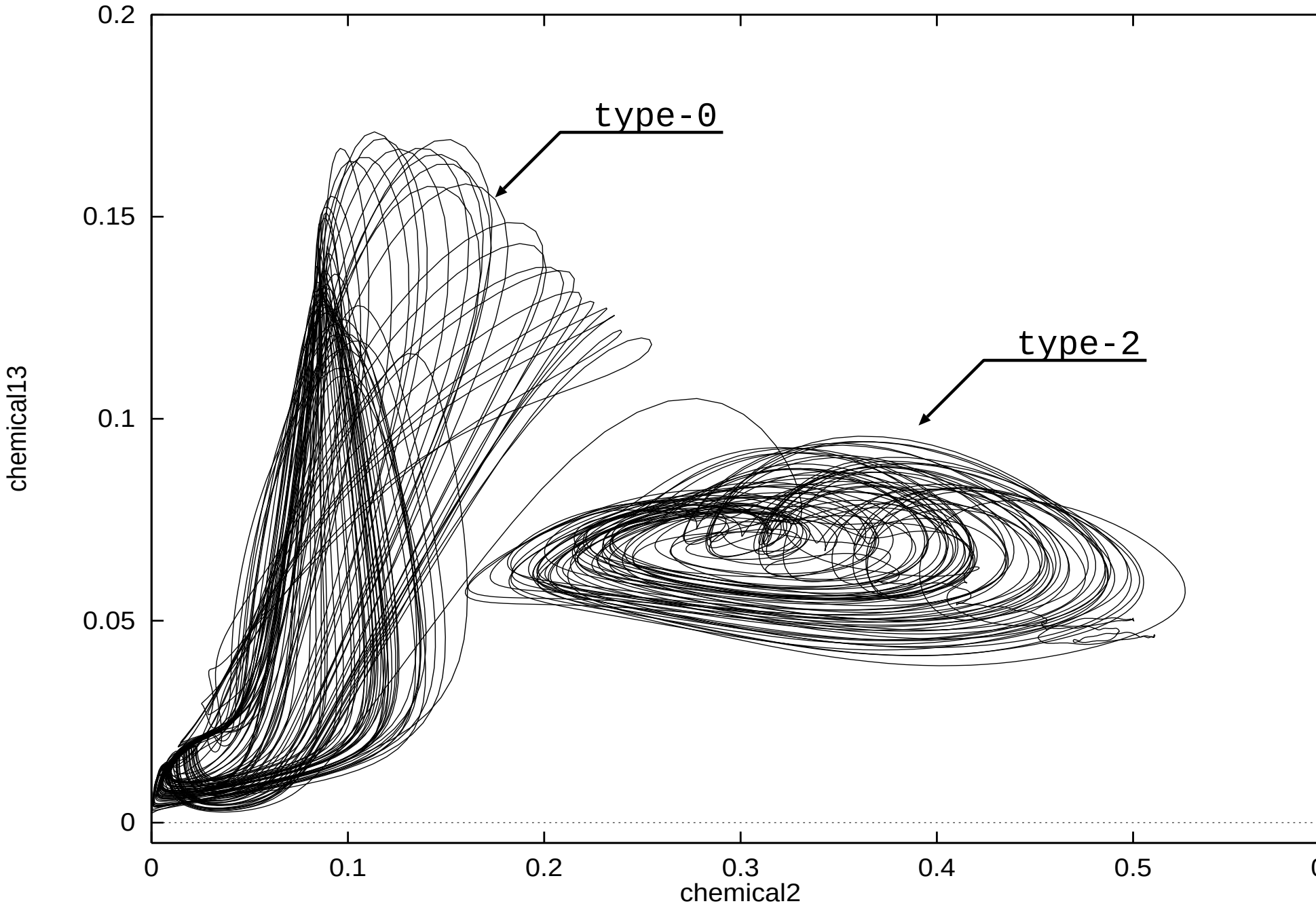
0.1
0
110000
115000
120000
125000
130000
135000
140000
145000
150000
155

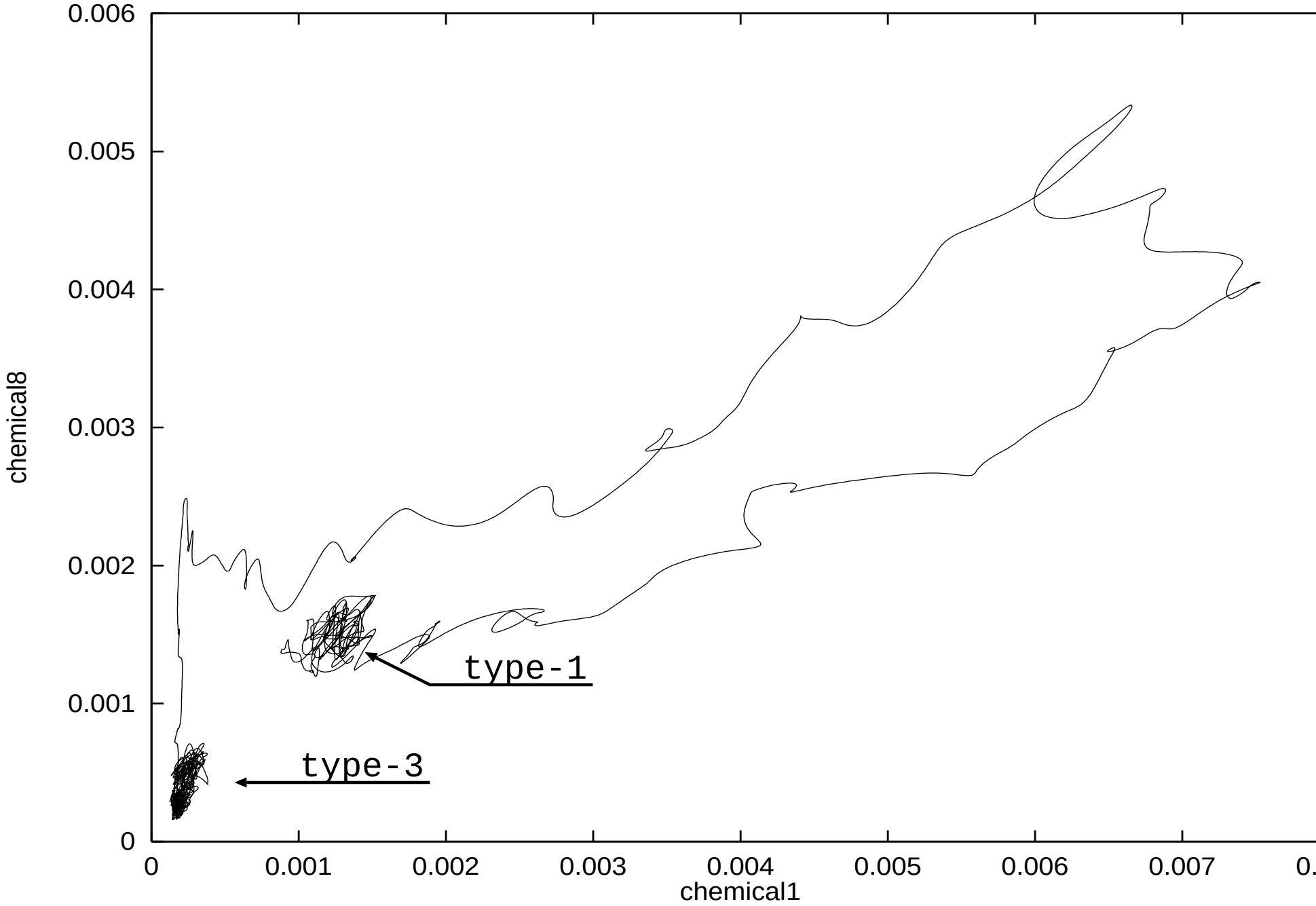

type-0
type-1
type-3
2
9
10
11
12
concentration
time
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
100000
110000
120000
130000
140000
150000
160000
170000
180000

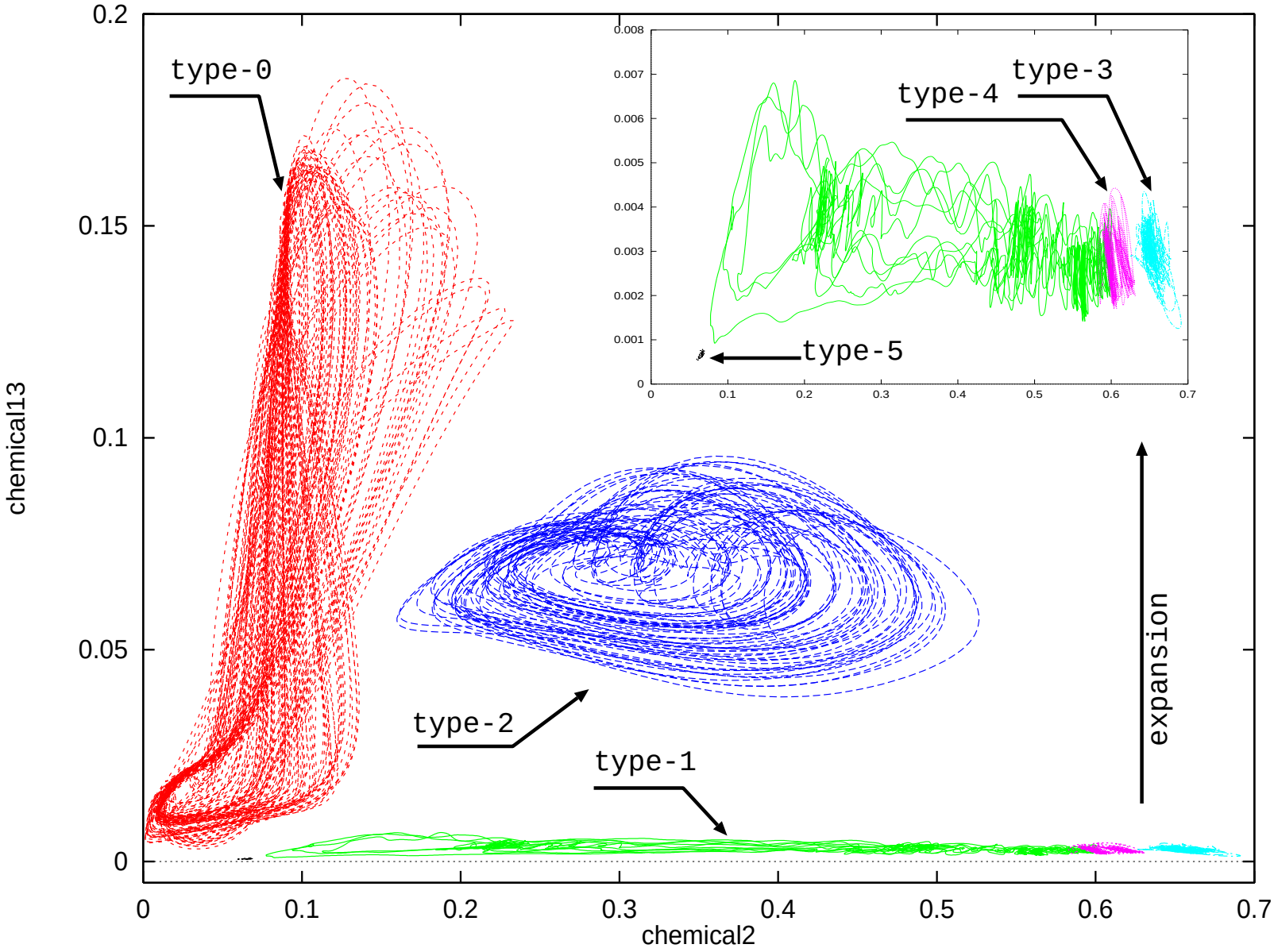

type-0
type-1
type-4
2
9
10
11
12
concentration
time
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
100000
110000
120000
130000
140000
150000
160000
170

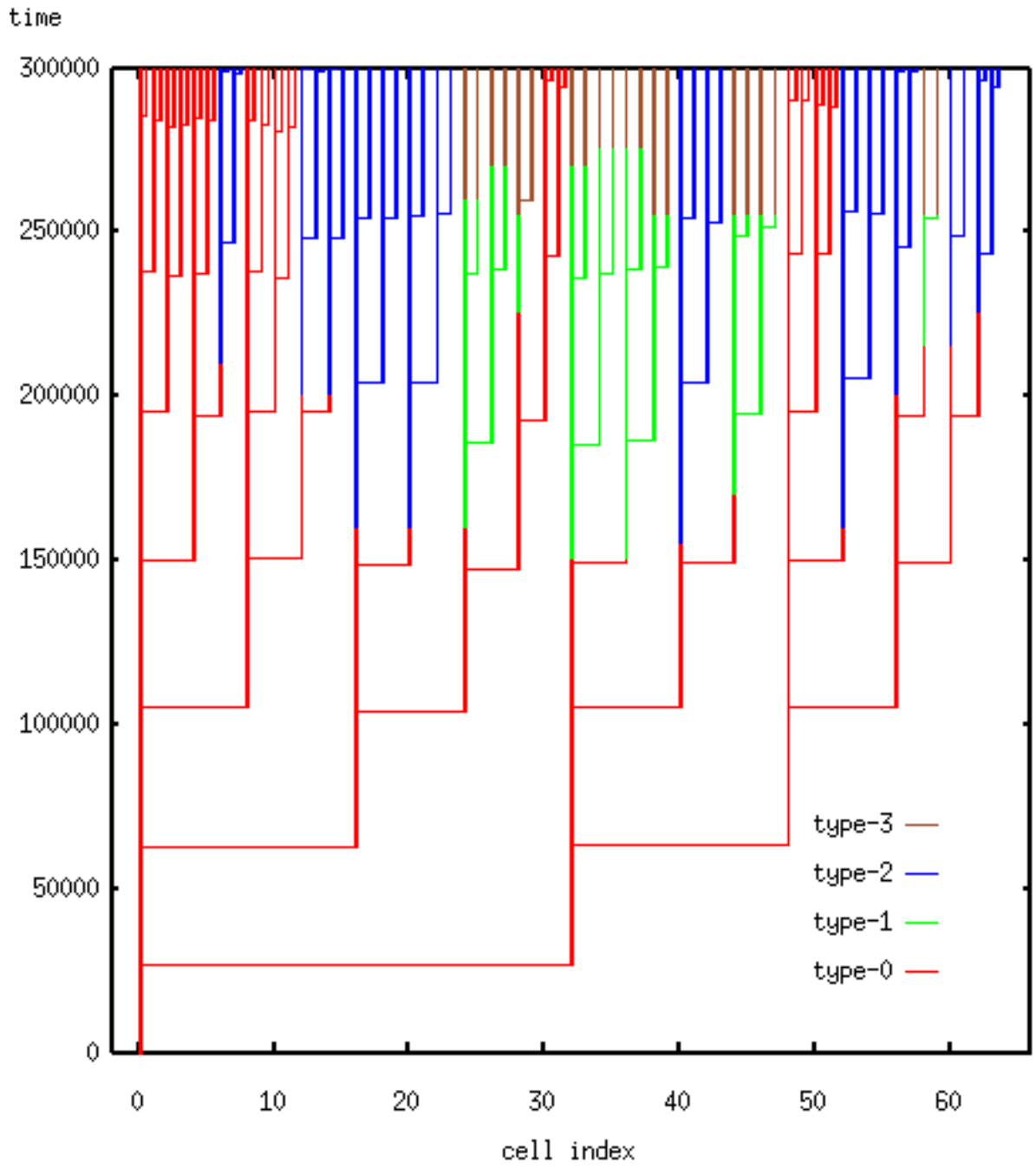

type-0
type-1
type-5
2
9
10
11
12
concentration
time
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
100000
150000
200000
250000
300000

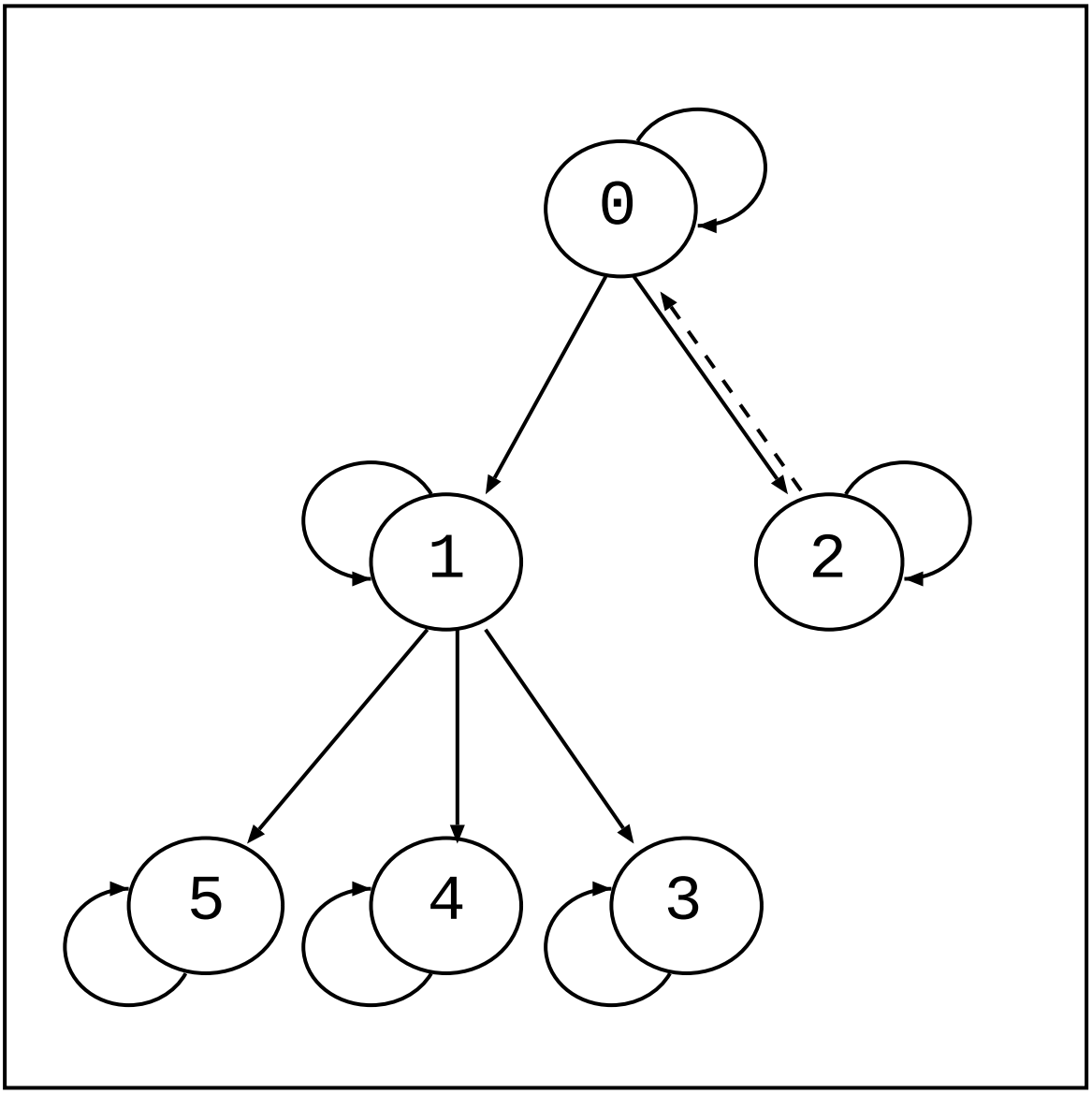

type-0
type-1
chemical13
chemical2
0.2
0.15
0.1
0.05
0
0
0.1
0.2
0.3
0.4
0.5
0.6

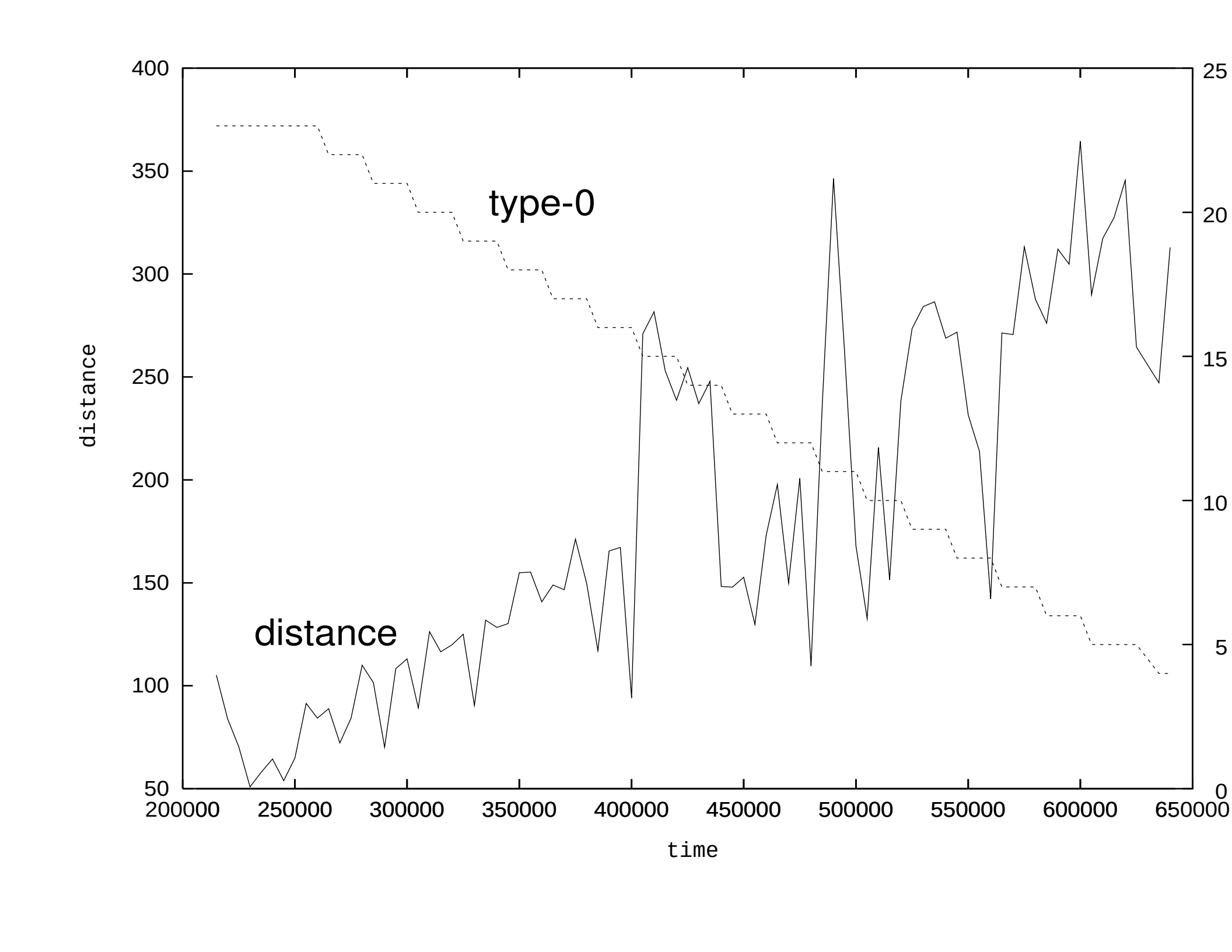

type-0
type-2
chemical13
chemical2
0.2
0.15
0.1
0.05
0
0
0.1
0.2
0.3
0.4
0.5

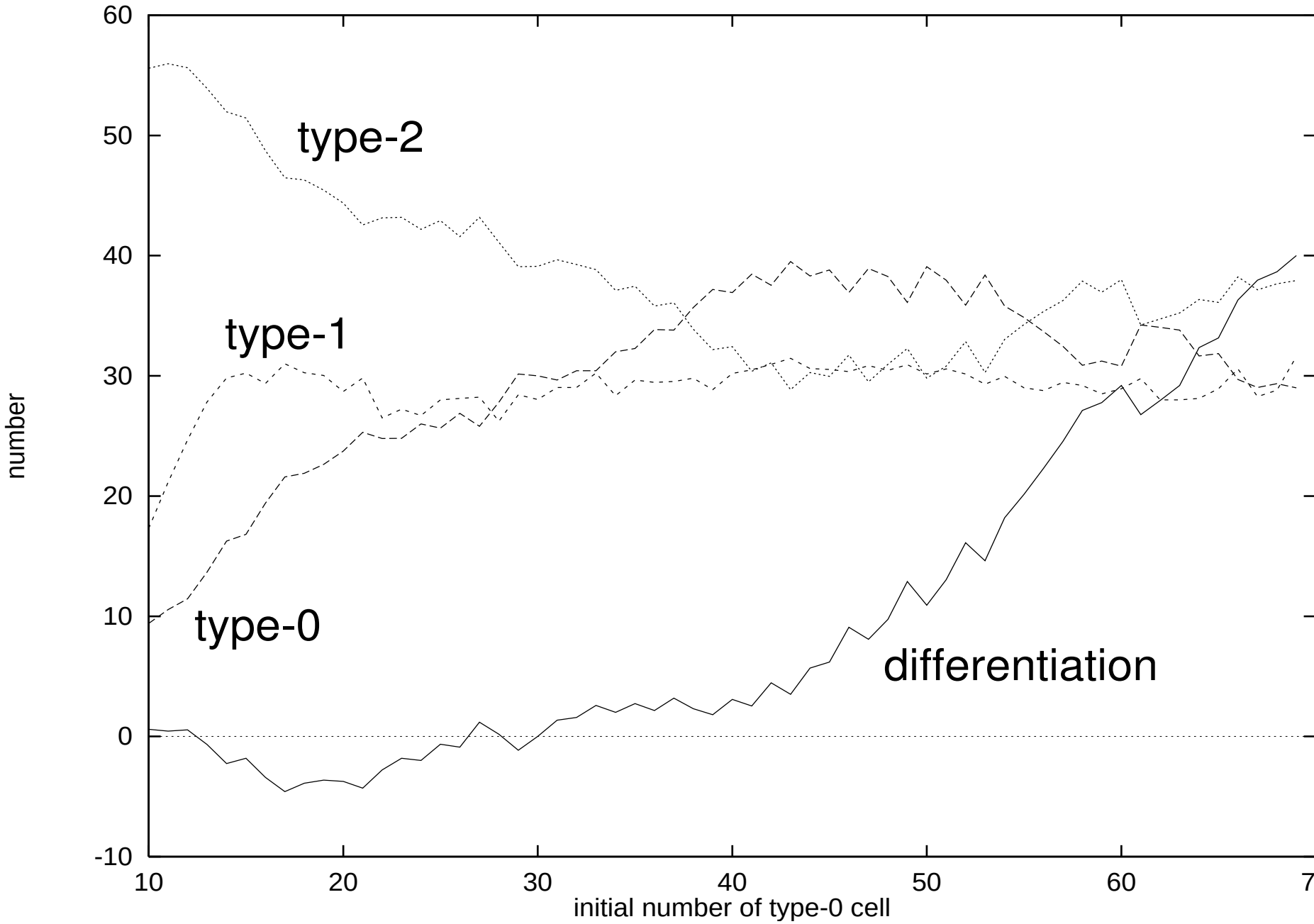

0.006
0.005
0.004
0.003
0.002
0.001
0
chemical8
0
0.001
0.002
0.003
0.004
0.005
0.006
0.007
chemical1
type-1
type-3

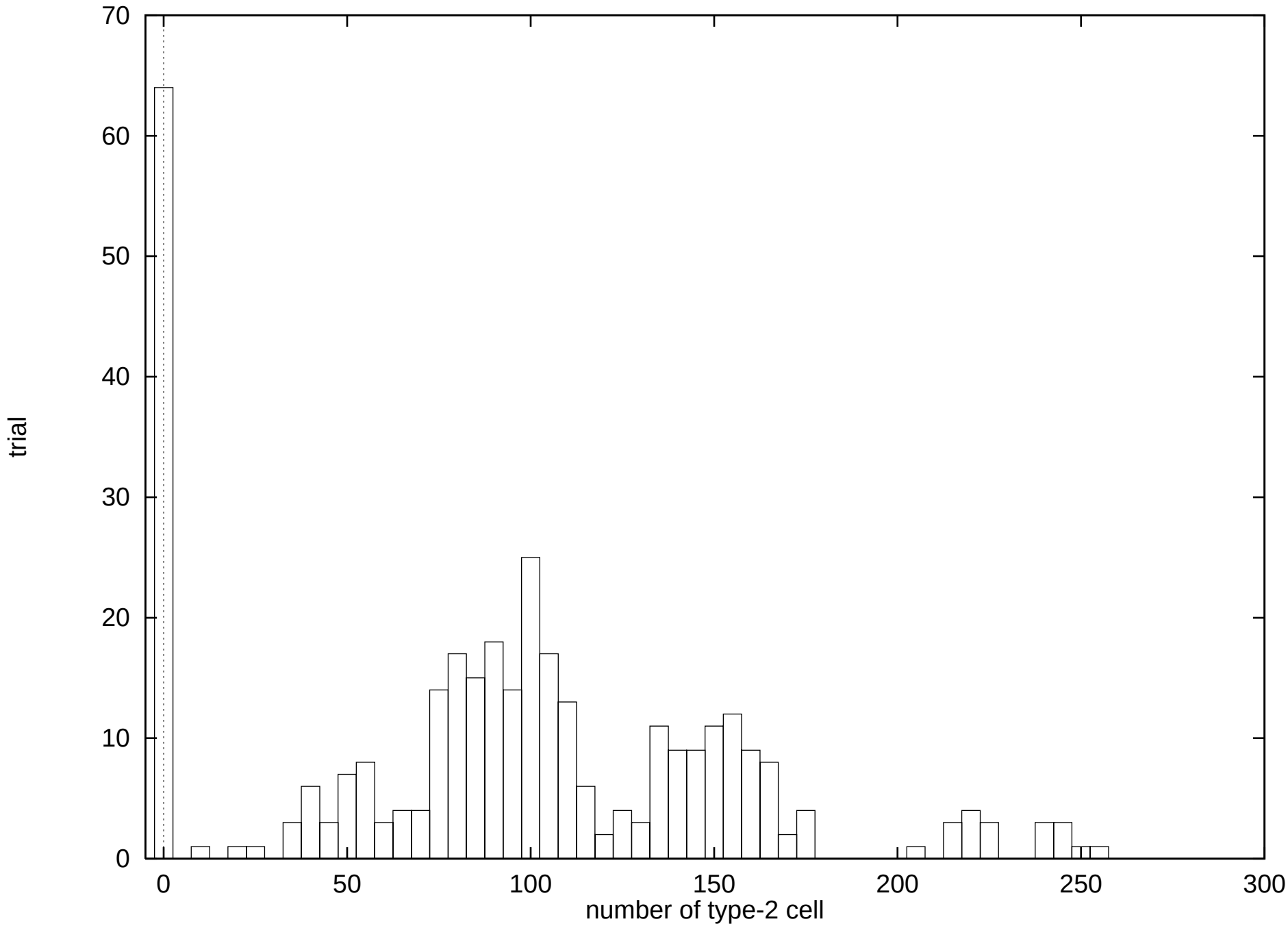

type-0
type-1
type-2
type-3
type-4
type-5
expansion
chemical13
chemical2
0.2
0.15
0.1
0.05
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001

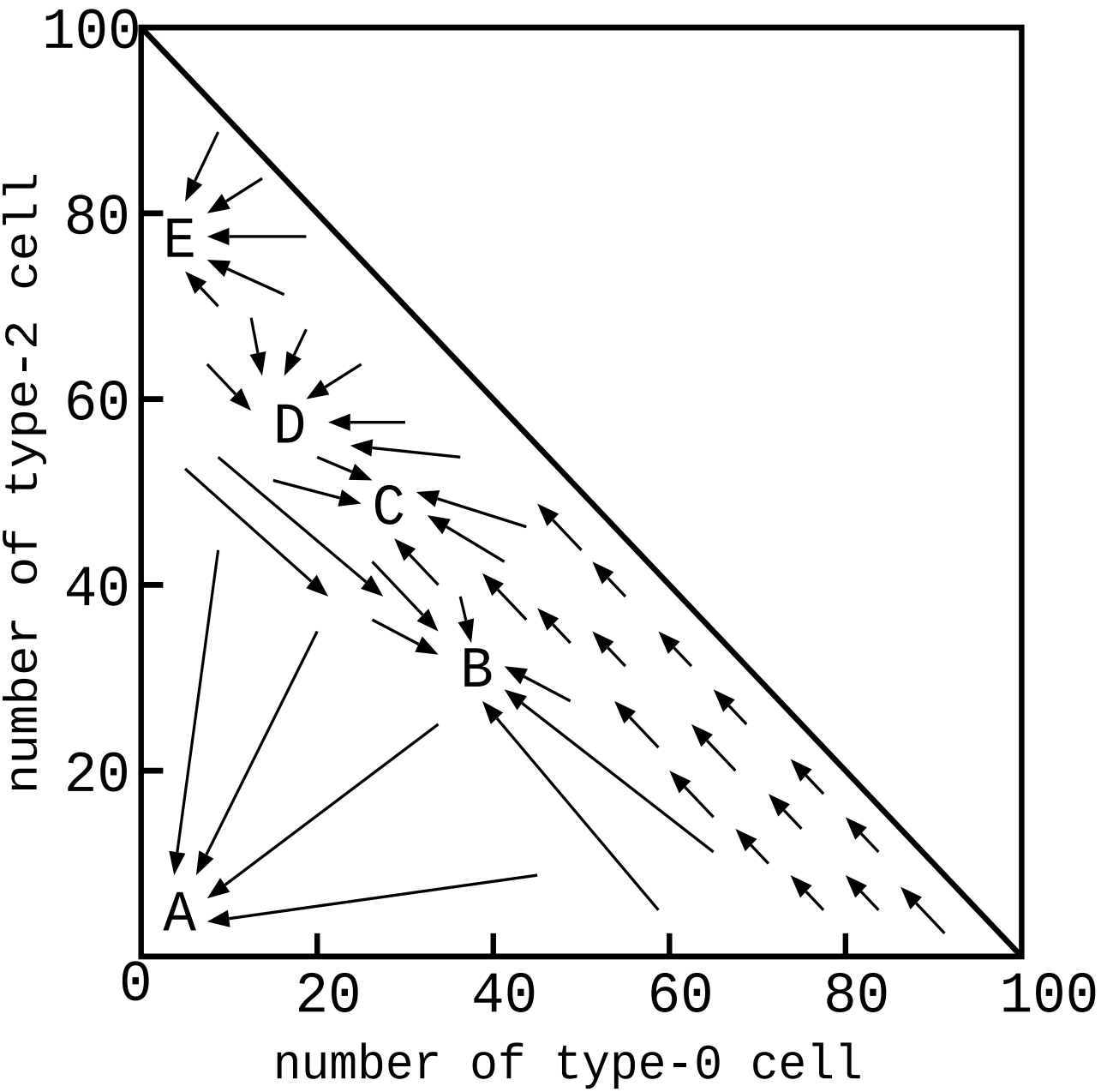

time
300000
250000
200000
150000
100000
50000
0
0
10
20
30
40
50
60
cell index
type-3
type-2
type-1
type-0


0
1
2
5
4
3


type-0
distance
distance
time
400
350
300
250
200
150
100
50
25
20
15
10
5
0
200000
250000
300000
350000
400000
450000
500000
550000
600000
650000


60
50
40
30
20
10
0
-10
number
type-2
type-1
type-0
differentiation
10
20
30
40
50
60
initial number of type-0 cell


70
60
50
40
30
20
10
0
trial
0
50
100
150
200
250
300
number of type-2 cell


100
80
60
40
20
0
20
40
60
80
100
number of type-2 cell
number of type-0 cell
A
B
C
D
E

	cell-0	cell-1	cell-2	cell-3	cell-4	cell-5
cell-0	1.3×10^{1}	1.8×10^{3}	1.6×10^{3}	2.9×10^{3}	3.0×10^{3}	3.2×10^{3}
cell-1	*	5.3×10^{2}	1.2×10^{3}	1.8×10^{3}	2.3×10^{3}	2.4×10^{3}
cell-2	*	*	2.0×10^{2}	1.5×10^{3}	1.5×10^{3}	3.6×10^{3}
cell-3	*	*	*	1.2×10^{1}	6.3×10^{2}	4.1×10^{3}
cell-4	*	*	*	*	2.3×10^{1}	4.6×10^{3}
cell-5	*	*	*	*	*	1.3×10^{1}

	cell-0	cell-1	cell-2	cell-3	cell-4	cell-5
cell-0	*	2.8×10^{2}	1.1×10^{2}	1.2×10^{3}	1.2×10^{3}	1.1×10^{3}
cell-1	*	*	1.2×10^{2}	8.0×10^{1}	8.7×10^{1}	2.8×10^{2}
cell-2	*	*	*	4.1×10^{2}	4.8×10^{2}	2.6×10^{3}
cell-3	*	*	*	*	4.9×10^{1}	2.6×10^{3}
cell-4	*	*	*	*	*	2.9×10^{3}
cell-5	*	*	*	*	*	*